University of Dundee

DOCTOR OF MEDICINE

Personalising Inhaled Corticosteroid Dose Response in Persistent Asthma

Anderson, William James

Award date:
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William James Anderson

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September 2016

For Kathryn, Louise and Luke

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ACKNOWLEDGEMENTS

This thesis would not have been possible without the help and support of numerous colleagues, friends and family. I therefore extend my sincere thanks to them, in particular:

Professor Brian Lipworth, my postgraduate supervisor, whose continuous guidance, encouragement, intellect, organisation and enthusiasm for clinical research was invaluable to me both academically and personally.

Dr Philip Short, my predecessor as clinical research fellow, for his guidance and support upon becoming a clinical research fellow, and his teamwork on designing, running and completing the propranolol study.

Dr Peter Williamson, a prior clinical research fellow, for his guidance and support in study design and clinical interpretation for both the FeNO and propranolol studies.

Dr Arvind Manoharan, my successor as a clinical research fellow, for his continued help with study recruitment and completion of the propranolol study.

Dr Tom Fardon, my secondary research supervisor, for his guidance and candor during my research, and for his critical review of this thesis.

Ms Patricia Burns, clinical trials manager, for her continued guidance through study approvals processes and data management.

Mrs Ashley Morrison, Ms Marney Keillor and Ms Carla Hogg, clinical research assistants, for their dedication and help with study recruitment, carrying out study visits, and collating study data.

Ms Kara Robertson, personal assistant to Professor Lipworth, for her help and organisation with administrative duties.

Mrs Lesley McFarlane, laboratory technician, for her efficient processing of study samples, and guidance about the results.

To all the study volunteers who willingly participated in the clinical trials.

To the Chief Scientist Office, Scotland for their research funding of the propranolol study.

Finally, but in no small measure, to my family – my wife Kathryn, my daughter Louise and my son Luke – without whose emotional support, love and understanding this period of research would not have been a success.

DECLARATION

I declare that I am the author of this thesis, with all references consulted by me, and I have generated all the findings in this thesis. The work described here has not been previously submitted or accepted for another higher degree. My contribution to the totality of work described here is as follows:

The majority of this work was carried out during my time as a clinical research fellow with the Asthma and Allergy Research Group, Division of Cardiovascular and Diabetes Medicine, Medical Research Institute, University of Dundee under the supervision and tutelage of Professor Brian Lipworth. The completion of this work was carried out when I became a consultant respiratory physician.

I took over as clinical research fellow in July 2011 from Dr Philip Short and Dr Peter Williamson, initially helping to complete some of their studies as well as commencing my own, as per the ethos of the department. Dr Arvind Manoharan also continued to recruit to my studies when my period of employment as a clinical research fellow ceased in July 2012, albeit my input continued once I then became a consultant physician in the same hospital with the aid of funding for dedicated research time from the Chief Scientist Office.

The FeNO study was conceived, designed and commenced by Professor Lipworth and Dr Williamson. My role was in completing study recruitment, personally analyzing all of the data, and preparing all versions of the manuscript leading to peer reviewed publication[1].

I analyzed the stored departmental data from a previously published study[2] by Dr Karine Clearie, a previous research fellow, in order to explore the hypothesis of obesity influencing ICS dose response. I prepared all versions of the manuscript leading to peer reviewed publication[3].

The propranolol study was the second part of a project grant awarded to Professor Lipworth and Dr Williamson by the Chief Scientist Office, Scotland; with the first study completed and published by Dr Short[4] (to which I also helped complete recruitment). I was integral to the study design for this second trial along with Professor Lipworth, Dr Short and Dr Williamson. I sought all the approvals, arranged all the blinded, randomised treatments, carried out the study, analysed all the data, and prepared all versions of the manuscript leading to peer reviewed publication[5].

The fourth study presents re-examined stored departmental data from screening episodes into a previously published clinical trial[6]. Furthermore, stored samples of bone markers were defrosted and analysed from this same prior trial for the final study presented in this thesis. I personally analysed all the data, and prepared all versions of the manuscripts leading to peer reviewed publications[7,8].

The fifth study pooled data from my own work and other previously stored data on ICS dose ramps from several previous clinical trials in the department. I personally analysed all the data in this new dataset, and prepared all versions of the manuscript for peer reviewed publication.

William James Anderson

SUMMARY STATEMENT

This thesis examines the overarching theme of inhaled corticosteroid (ICS) dose response effects on a variety of asthma outcome measures; with further importance placed on the application of these findings to personalising ICS dosing for the individual asthmatic.

The introduction provides a detailed summary of the current recommendations for the treatment of adult asthma, with particular reference to the mechanism of action and clinical utility of ICS for the treatment of asthma. Current methods of assessing ICS dose response are presented, as well as the common influences that affect these responses. Novel therapeutic theories and the identification of specific asthmatic phenotypes are also introduced, in order to demonstrate the shift towards personalising treatment for asthma.

The first two studies examine the dose response of ICS on two specific factors that influence asthma. The third study presents an examination of pharmacological manipulation of the ICS dose response using an additional agent. The following two studies address: how asthma outcomes relate to each other in patients receiving ICS; in addition to an overall assessment of the ICS dose response across a broad range of both ICS moieties and outcome measures. The final study examines for any detrimental effect of an ICS dose ramp on bone metabolism, an important potential long-term adverse effect of higher ICS dosing.

The discussion draws together all the results obtained in relation to ICS dose response in asthma, and how these apply to current clinical practice for the individual patient.

Furthermore, hypotheses are generated for areas of future study based on the findings from this work.

LIST OF ABBREVIATIONS

ABPA	Allergic bronchopulmonary aspergillosis
ACD	Asthma control days
ACQ	Asthma Control Questionnaire
ACT	Asthma Control Test
ACTH	Adrenocorticotrophic hormone
AHR	Airway hyper-responsiveness
AIRE	Asthma Insights and Reality in Europe study
AMP	Adenosine monophosphate
AMPUL	Asthma Management Project University Leiden group
ANOVA	Analysis of variance
AQLQ	Asthma Quality of Life Questionnaire
ARIA	Allergic Rhinitis and its Impact on Asthma
ATS	American Thoracic Society
Ax	Area under the curve
B-17-MP	Beclometasone-17-monopropionate
B2ADR	Beta-2 adrenoceptor
BDP	Beclometasone dipropionate
BMI	Body mass index
BP	Blood pressure
BTS	British Thoracic Society
Bud	Budesonide
CFC	Chlorofluorocarbon
CI	Confidence interval
CIC	Ciclesonide
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic obstructive pulmonary disease
CTx, 1CTP	C-terminal cross-linked telopeptides of type 1 collagen
CV	Coefficient of variation
DALY	Disability adjusted life year
des-CIC	Desisobutyryl-ciclesonide
DPI	Dry powder inhaler
ECP	Eosinophilic cationic protein
Eos	Eosinophils
ERS	European Respiratory Society
EVH	Eucapnic voluntary hyperventilation
FEF_{25-75}	Forced expiratory flow between 25-75% of FVC
FeNO/NO	Fractional exhaled nitric oxide / nitric oxide
FEV_1	Forced expiratory volume in 1 second
FEV_6	Forced expiratory volume in 6 seconds
FF	Fluticasone furoate
FP	Fluticasone propionate
F_{res}	Resonant frequency
FVC	Forced vital capacity
GCP	Good Clinical Practice

GINA	Global Initiative for Asthma
GMFD	Geometric mean fold difference
GOAL	Gaining Optimal Asthma Control study
GORD	Gastro-oesophageal reflux disease
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
HDAC2	Histone deacetylase 2
HFA	Hydrofluoroalkane
HPA	Hypothalamic-pituitary-adrenal (axis)
HR	Heart rate
HRQoL	Health related quality of life
ICS	Inhaled corticosteroid
IgE	Immunoglobulin E
IL	Interleukin
IMPACT	Improving Asthma Control study
IOS	Impulse oscillometry
LABA	Long acting beta-2 agonist
LAMA	Long acting muscarinic antagonist
LTRA	Leukotriene receptor antagonist
MANOVA	Multivariate analysis of variance
MCID	Minimum clinically important difference
MF	Mometasone furoate
MHRA	Medicines and Healthcare Regulatory Authority
MMAD	Mass median aerodynamic diameter
NF-κβ	Nuclear factor - kappa beta
NIH	National Institutes of Health
NRAD	National review of asthma deaths
OR	Odds ratio
OUCC	Overnight urinary cortisol/creatinine ratio
PC_{20}	Provocative concentration causing 20% fall in FEV_1
$PD_{10,15,20}$	Provocative dose causing 10, 15 or 20% fall in FEV_1
PEF/PEFR	Peak expiratory flow / rate
pMDI	Pressurised metered dose inhaler
P1NP	Procollagen type 1 N-terminal propeptide
P3NP	Procollagen type 3 N-terminal propeptide
Ppb	Parts per billion
R20	Airway Resistance at 20 Hertz
R5	Airway Resistance at 5 Hertz
R5-R20	Airway Resistance between 5 and 20 Hertz
RCP	Royal College of Physicians
RCT	Randomised Controlled Trial
SABA	Short acting beta-2 agonist
SPT	Skin prick test
STAMINA	Steroid Titration Against Mannitol in persistent Asthma study
TH2	T-helper 2
TNF-α	Tumour necrosis factor alpha

CHAPTER 1:

INTRODUCTION AND

LITERATURE REVIEW

DEFINITION AND EPIDEMIOLOGY OF ASTHMA

'Asthma is a heterogeneous disease, usually characterised by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory flow limitation.' This is the consensus statement on the definition of asthma from the Global Initiative for Asthma (GINA) guidelines[9]. There is a similar theme to the statements defining asthma from both the British Thoracic Society (BTS)[10] and American Thoracic Society (ATS)[11] by way of confirming that the diagnosis of asthma is largely a clinical one for which there is no gold standard threshold for accurate identification of the disease. Indeed they affirm the common strands of pulmonary symptomatology with variable airflow obstruction, along with perhaps the additional identification of airway hyperresponsiveness (AHR, higher sensitivity/twitchiness to stimuli) and airway inflammation upon patient testing – considered the two key hallmarks of the underlying pathophysiology of asthma[12]. Indeed both of these features of asthma have been consistently shown to improve with inhaled corticosteroid (ICS) treatment[6,13-15], which I will discuss in more detail later in this introduction.

It is vitally important that we correctly diagnose asthma in an individual so that we can approach their treatment options effectively. Whilst this is an obvious statement, it is often difficult to achieve in practice. The symptomatology of asthma is widely variable, both in terms of frequency of occurrence and in the total number and severity of symptoms any given individual might display[16,17]. Furthermore, simple tests for airflow obstruction in asthma are widely available – including serial peak expiratory flow rate (PEFR) measurements and spirometry – but their predictive value

in diagnosing asthma is only moderate, or worse if trying to identify mild disease[10,11,18,19]. Bronchial provocation testing as a measure of airway hyperresponsiveness; and level of exhaled nitric oxide as a measure of airway inflammation, have also been used to try to increase the accuracy of asthma diagnosis[20-22]. However, these investigations are by no means perfectly diagnostic of asthma and still remain largely limited to university hospitals and research facilities, due to their complexities of administration and interpretation, and have not therefore made it into widespread primary care use at the present time. Ultimately when one is tasked with diagnosing asthma, a combination of symptoms and airway measurements are required to assist in determining the probability that any individual does actually suffer from it. Indeed helpful decision making trees and probability tables have been included in the most recent iteration of the BTS asthma guidelines[10].

Asthma is recognised to be a condition of international importance and impact. The Global burden of Asthma document[16] developed for the Global Initiative for Asthma (GINA) has not been updated since 2004. Nevertheless, even at that time there was a conservative estimate that 300 million people in the world were suffering from asthma – equating to approximately 5% of the total population of the world. However, this worldwide prevalence of clinical asthma is not evenly distributed, with higher rates of asthma apparent with increasing 'westernisation' of lifestyle and urbanisation of communities. Indeed, the mean prevalence of clinical asthma in the UK and Republic of Ireland in 2004 was 16.1% - one of the highest in the world. Given that the prevalence of asthma continues to increase, this may well equate to an additional 100 million people suffering from asthma worldwide by 2025[16].

Not only is the prevalence of asthma significant, there is also an attendant economic burden as well as significant morbidity and mortality associated with the disease. Asthma was the 25th leading cause of disability-adjusted life years (DALYs) lost worldwide in 2001 at 15 million – similar to the impact of diabetes and liver cirrhosis[16]. In the UK, asthma care and services cost the NHS almost £900 million (accounting for both hospital services and prescribed medication costs), and asthma is responsible for at least 12.7 million lost working days every year (Asthma UK).

Most concerning of all is that there remains an associated mortality with asthma, and this despite it being an eminently treatable condition, with all deaths from asthma being theoretically preventable for this reason. Previous confidential enquiries into asthma deaths in Scotland[23] and Wales[24], both between 1994-1996, identified a variety of reasons for this: including inappropriate routine prescribing, patient behaviours and circumstance, as well as disease severity; albeit in the Scottish report there had been an overall improvement since the early 1980's. Fast-forward to 2014 when the Royal College of Physicians delivered their National Review of Asthma Deaths (NRAD) confidential enquiry report[25], which, despite the findings of these historic reports, still makes for sobering reading regarding ongoing, preventable asthma deaths.

The Royal College of Physicians in their NRAD report presented key findings in four main areas: NHS services; medical and professional care; prescribing and medicines use; as well as patient factors and perception of risk of poor control. It found that 57% of those who died were not under specialist asthma care. Worryingly, only 39% of patients who died were actually labelled as having 'severe' asthma according to

current guidelines, i.e. the remainder were labelled as either 'mild' or 'moderate' – suggesting we still cannot accurately identify those at greatest risk. Patient factors were again highlighted, such as persistent smoking exposure, non-attendance for clinic review and poor recognition of the risk of not having good asthma control perhaps for psychological or psychiatric reasons. Furthermore, and particularly germane to this thesis, are the findings of over-prescription of asthma reliever medication *in addition to* under-prescription of preventer medication such as ICS in patients who died. Indeed it has previously been shown that regular usage of ICS in asthma can both prevent hospitalization over the long-term[26] as well as prevent asthma deaths[27]. Moreover, suboptimal asthma control has been shown to increase the likelihood of having an asthma exacerbation in the future[28,29]. A large European survey of 8000 asthma patients in primary care[30] concluded that asthma control remains poor, with symptoms and exacerbations commonly seen – yet many patients felt that despite this their asthma could be considered 'controlled'. This apparent lack of insight into the risks associated with uncontrolled disease by patients, had previously been demonstrated in another large European study in 1999 by Rabe *et al* – the Asthma Insights and Reality in Europe (AIRE) study[31].

While great strides have been made to improve asthma care over the past 20-30 years, we are not there yet. We still cannot completely and accurately identify who has asthma and who does not. Furthermore, we are still under-prescribing (and patients are underusing) the most beneficial treatment for asthma – namely ICS – along with inappropriate usage of other asthma medications, such as over-reliance on rescue inhaled bronchodilator therapy. We therefore need to better identify: who to treat, taking into account the variation of intrinsic pathophysiology as well as external

factors (e.g. obesity); and what the optimal medication strategy is for a given individual. I aim to improve clarity on some of these issues with specific regard to the mainstay of asthma treatment - inhaled corticosteroid therapy - in the remainder of this thesis.

CURRENT PHARMACOLOGICAL TREATMENT RECOMMENDATIONS FOR PERSISTENT ASTHMA

Once the diagnosis of asthma has been made in an individual, we need to consider what (level of) treatment will be required, with the ultimate aim of gaining complete control of their asthma symptoms, thereby reducing the risk of asthma exacerbations. Complete control as defined by the BTS includes: no daytime symptoms, no night-time awakening due to asthma, no need for rescue medication, no asthma exacerbations, no limitations on activity including exercise, normal lung function, and minimal side effects from medication[10]. For the remainder of this section I will be discussing the current pharmacological treatment recommendations pertaining to *adults* with asthma, as the studies in my thesis all pertain to adult persistent asthmatics over 18 years of age.

The BTS advocate a stepwise approach to the pharmacological management of asthma. Progression upwards through the treatment steps from 1-5 is aligned to worsening symptomatology as well as the sequelae of those symptoms, i.e. excess reliever use, oral steroid use, and exacerbations. There is certainly evidence that when guideline determined symptom control is used stringently (as part of a randomised controlled clinical trial) for altering the type and dosing of inhaled asthma therapies, then significant overall improvements in asthma control are seen. For example, in the 'GOAL' study, patients were managed according to GINA/National Institutes of Health (NIH) guideline targets for totally controlled and well controlled asthma[32]. By using this approach, the majority of patients improved from being uncontrolled to being controlled according to the guideline definitions. The BTS have included in their guideline a choice of symptom-based questionnaires to be used for

this purpose. Not all have been well validated, however. Those that have been well validated for assessing asthma control include the Asthma Control Questionnaire (ACQ)[33,34] and the (Mini)-Asthma Quality of Life Questionnaire (AQLQ)[35] (Appendix II – asthma questionnaires). Indeed, in a post-hoc analysis of the GOAL study by Meltzer and colleagues[29], ACQ was the strongest predictor of a future exacerbation. Oddly, the BTS seem to favour the use of a not well-validated questionnaire, namely the Royal College of Physicians (RCP) 3 questions[36], purely on the basis that it may be simpler to use, without knowing whether or not it is the better test. Moreover, it is not at all clear how well these recommendations are adhered to in the UK, in terms of optimising asthma therapy, and particularly in primary care where the majority of asthma patients are cared for and where the bulk of the recommendations apply. However, where this has been examined in other countries of similar socioeconomic status to the UK, for example Israel[37] and Kuwait[38], asthma guideline adherence rates in primary care have been found to be low.

Mild intermittent asthma is at the bottom rung of the BTS asthma treatment ladder (Figure 1) at step 1, where symptoms are experienced less than once per week, and patients are generally treated with an ‘as required’ inhaled short-acting Beta-2 agonist (SABA), usually salbutamol. SABAs cause rapid bronchodilation of the airways through relaxation of the airway smooth muscle upon activation of their surface beta-2 adrenoceptors (B2ADRs), and thus are ideal for symptom control in asthma. However, they do nothing to counteract the underlying inflammatory processes that cause an individual to have hyper-responsive airways, which then constrict when exposed to inhaled stimuli.

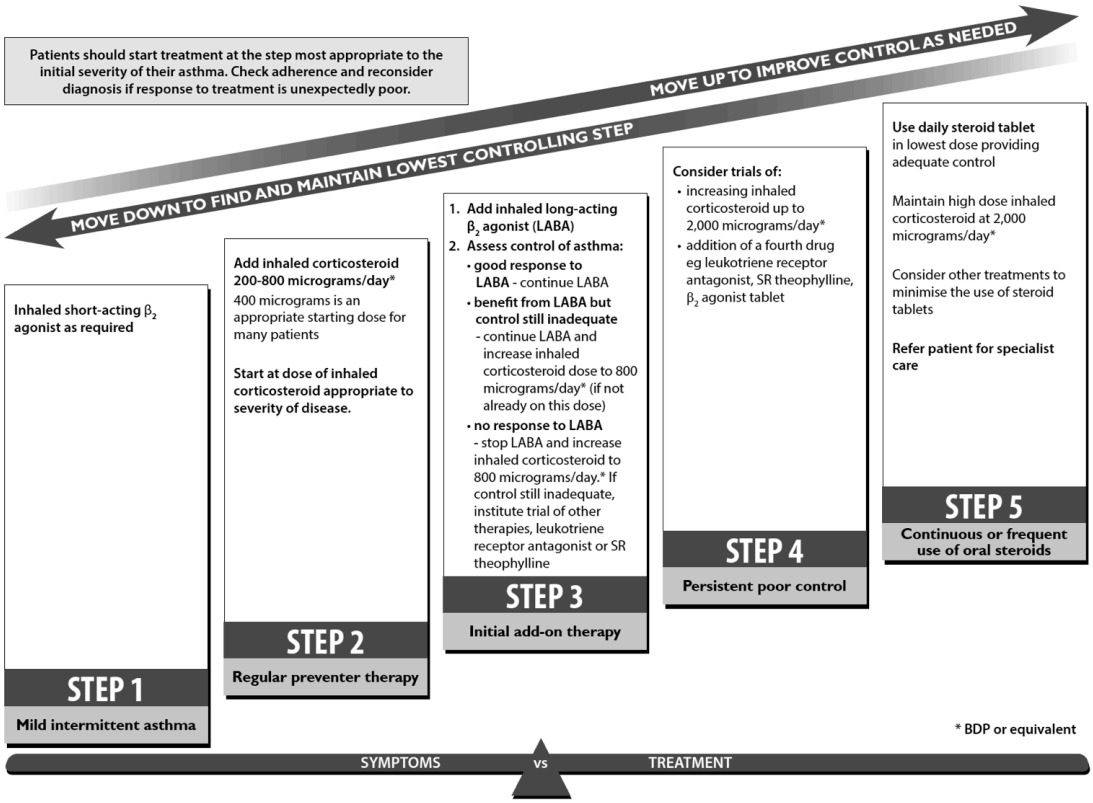

Figure 1. Summary of stepwise asthma treatment titration from BTS/SIGN asthma guidelines 2014.

The persistence of mild asthma symptoms heralds step 2 of asthma treatment. It has been shown that even in mild asthma, there is ongoing airway inflammation[39]. Furthermore as symptoms, and therefore inflammation, become more persistent, there is an increased risk of exacerbation and death – even in those patients deemed to have mild disease[25]. It is for these reasons that ICS are recommended at this seemingly early stage. It is not surprising that ICS have become the backbone of asthma controller therapy due to their wide-ranging effects not only on asthma symptoms, but also on their ability to control the underlying inflammatory process that heralds these symptoms in the first place. They have been shown to reduce asthma symptoms[40-42], improve lung function[43,44], decrease the frequency and severity of exacerbations[45-47], as well as improve quality of life[48]. Moreover, the therapeutic index of ICS for

treating mild asthma is favourable, as asthma control can usually be achieved with low doses in this group, thus negating any significant local or systemic side effects[49].

Some uncertainty does emerge when treating mild asthma, due to the ongoing argument as to whether or not mildly symptomatic patients should receive continuous or intermittent ICS therapy. The argument for intermittent ICS use is largely borne out of a widely demonstrated lack of compliance to regular inhaled medications, perhaps because in mild asthma the symptom burden is insufficient to reinforce the need for regular therapy. Boushey *et al* prospectively examined this question in the Improving Asthma Control (IMPACT) study[50]. They compared three groups of mild asthma patients, randomising them to one of: regular budesonide, regular zafirlukast (a leukotriene receptor antagonist), or intermittent budesonide to be used when asthma symptoms occurred – all in a randomised, placebo-controlled fashion. They found no difference in their main outcome measure of change from baseline in two-week average morning PEF, with similar small increases across the groups. Furthermore, there was no difference in the number of exacerbations across the groups over the one-year follow-up period. However, they did observe improvements in inflammatory outcomes, namely sputum eosinophilia and exhaled nitric oxide (FeNO), in the regular budesonide group, but not in either of the other two groups. This is important, as previous studies have reported that regular ICS treatment may prevent progressive loss of pulmonary function[51,52] and chronic airway remodelling which might be due to unopposed ongoing airway inflammation[39]. Pointedly, we have already seen that an approach of regular ICS therapy reduces both frequency of exacerbations[26] and mortality[27]. Nevertheless, the authors of the IMPACT study do concede that whilst an intermittent ICS treatment strategy may be viable, it cannot yet be recommended on the basis of the current available evidence, which remains the

standpoint of most recent guidelines[10,11].

It is important to also mention at this juncture that there is no role for long-acting beta-2 agonists (LABAs) as single-agent asthma controller therapy, without ICS. There is wide agreement in the literature that LABA monotherapy should be avoided in asthma. This is on the basis of studies that have shown significant adverse events and mortality using this approach[53-58]. The main reasons given are: over-reliance (on beta-2 agonists) and under-treatment (with ICS) masking inflammation and severity of disease; tolerance to treatment; and increased airway hyper-responsiveness to allergens. Beta-2 agonists may also activate non-canonical (Gs-cAMP independent) Beta-arrestin mediated pro-inflammatory signaling pathways via extracellular signal regulated kinases (ERK1/2)[59]. In knockout mice Beta-arrestin-2 regulates the development of allergic asthma[60]. In the antigen driven mouse model, depletion of adrenaline - the natural ligand for the B2ADR - prevented the development of asthma[61]. Replacement of B2ADR signaling by administration of formoterol (LABA) restored the asthma phenotype, showing that agonist-induced activation of B2ADRs results in development of asthma. Therefore, giving ICS with LABA might obviate any pro-inflammatory effects of the LABA, while retaining the airway stabilizing activity of the LABA. It is therefore recommended that if a LABA is prescribed, this should always be in conjunction with background ICS therapy. There is much less agreement, however, on the optimum role of LABA prescribed in addition to ICS[62].

Moving onto step 3 of the BTS treatment ladder, we are now faced with a choice of treatment approaches when symptoms remain uncontrolled by regular low to moderate dose ICS. The two main strategies are to either increase the dose of ICS, or to add another asthma controller drug, which in the main is LABA therapy. There

have been a number of studies showing that adding LABA to ICS versus increasing the ICS dose alone is of greater therapeutic benefit[45,63,64]. Furthermore, it is recommended that ICS/LABA should be given in single combination inhalers where possible, to avoid any likelihood of inadvertent LABA monotherapy[10]. Albeit, a number of studies have concluded that there is no therapeutic advantage to using this single inhaler/dual therapy approach over concurrent LABA and ICS inhalers[65,66]. There are several other options for add-on therapy to ICS, including leukotriene receptor antagonists (LTRAs)[67], theophyllines[68] or slow-release beta-2 agonist tablets[69]; but these have not been as extensively studied in terms of asthma outcomes than when compared to adding LABA to ICS. While the evidence base behind the current BTS guidelines certainly advocates LABA/ICS combination before increasing ICS dose further, there is more recent evidence from a large 'real-life' study suggesting there might be no difference between these approaches in a broad primary care population[70]. Bateman et al. found that the odds of asthma control and rates of severe exacerbation over one outcome year were comparable between increased ICS dose versus added LABA groups. This is relevant because there may be bias in previous studies on the basis of their tight inclusion criteria, requiring both the presence of prior reversibility to SABA upon study entry and the use of lung function outcomes such as PEF that may have favoured the LABA component over ICS. Ultimately, I think that we need to concentrate on the individual patient presenting before us, to decide what the optimum treatment regime might be for them, particularly when randomised controlled trials and population statistics are conflicting.

We now move to the more severe end of the asthmatic spectrum at step 4. There are very few randomised controlled trials to inform how we approach management of

these patients, with extrapolation from trials of add-on therapies being the basis for any decisions. If patients are already on an ICS/LABA combination, with an ICS beclometasone dipropionate (BDP) equivalent dose of 800µg/day, then it is suggested any of: increasing ICS dose up to 2000µg/day, or adding in any of LTRAs, theophyllines or slow-release beta-2 agonist tablets – bearing in mind the greater side effect profiles of these latter two. Equally, at such high doses of ICS, then the risk of side effects from these increases too[49]. The long-acting muscarinic antagonist (LAMA) tiotropium has also been shown to be beneficial when added in here[71,72]. It is important to remember at all stages of asthma therapy and in particular at this level of treatment, one has to be assured of an adequate inhaler technique (with the use of a spacer device if necessary) as well as being quite ruthless in assessing patients' adherence to medication[73].

Finally we get to step 5 of the BTS asthma treatment ladder. These severe asthma patients who are refractory to standard medications arguably represent a completely different phenotype in asthma. However, the treatment options for these individuals, when it comes to maximizing anti-inflammatory treatment, are very limited. Furthermore, the role of comorbidities is greater in this patient group. Currently the BTS recommend using the lowest controlling dose of oral corticosteroids as the next step for patients uncontrolled at step 4. Clearly oral steroid side effects are much greater than that from ICS, and a variety of organ systems should be screened for these side effects when oral steroid treatment is either continuous, or courses are frequent[10]. For a small group of severe allergic asthmatics, there is the option of anti-IgE therapy – omalizumab – that is a monoclonal antibody that binds to circulating IgE and reduces serum IgE levels[74]. It can provide significant benefits in terms of reducing asthma exacerbations and the need for oral steroid therapy[75]. However, this

treatment is limited to patients who display a clear allergic component to their asthma (with either skin-prick or specific serum IgE positivity to allergen) as well as having an elevated serum total IgE level within pre-defined upper and lower limits for which the anti-IgE therapy can be used. It goes without saying that these patients should be assessed and treated in specialist centres (as should all step 5 patients). Because of the significant benefits seen in these carefully selected patients, GINA[9] have recently put omalizumab on equal footing with oral steroids as the joint first line choice for step 5 (in suitable patients) – a move which I believe is likely to be adopted by the BTS in the future, given its excellent side effect profile compared to oral steroids. Another monoclonal antibody, the anti-IL5 agent mepolizumab, has recently become available for patients with severe eosinophilic asthma[76]. Indeed blood eosinophil levels are receiving more attention once again in asthma due to their effects on increased likelihood of exacerbation[77] and because they can be therapeutically targeted by mepolizumab through its anti-IL5 actions. Other steroid-sparing agents (e.g. methotrexate) can be used but there is little evidence for them in asthma per se.

Overall, ICS remain the cornerstone of asthma controller therapy throughout all but the first asthma treatment step. Work is still needed to accurately identify when and in whom ICS therapy should be altered, the doses required to completely control both symptoms and underlying airway inflammation, as well as exploring novel add-on therapies or ICS sparing agents.

PHARMACOLOGY OF INHALED CORTICOSTEROIDS

General immunosuppressive effects

Inhaled (gluco-) corticosteroids are the most valuable group of drugs for combating the widespread inflammatory effects in asthmatic airways. As such they have revolutionised the way we treat asthma, in particular enabling the reduction in need for regular oral corticosteroids[78] with their significant attendant side effect profile. Corticosteroids exert their anti-inflammatory effects through a variety of mechanisms[79]. They both increase and decrease different gene transcription pathways in the cell nucleus that are involved in the pathological processes of asthma, largely to decrease the overall level of inflammation as well as exerting effects on other receptors. These include genes encoding for: B2ADRs, anti-inflammatory cytokines (e.g. IL-10, IL-12), inflammatory cytokines (e.g. IL-2 to IL-6, IL-13, TNF-α), inflammatory enzymes (e.g. inducible nitric oxide synthase [iNOS], inducible cyclooxygenase [COX-2]), adhesion molecules (e.g. ICAM-1), and many others. Furthermore, they reduce the numbers of a variety of inflammatory cells including eosinophils, T-lymphocytes, dendritic cells, macrophages and mast cells along with reducing the levels of cytokines and other inflammatory mediators that drive their production. Corticosteroids additionally have effects on restoring endothelial cell integrity and reducing cytokine production from these cells – this is important, as the airway epithelial cells are the main targets for ICS. Moreover, corticosteroids have anti-proliferative properties[80] that they exert on airway smooth muscle, particularly as smooth muscle airway proliferation leads to increased AHR and potentially airway remodelling in asthma. Most of these effects occur rapidly, for example, a visible reduction in the level of eosinophils can be seen within 6 hours[81].

Effects on glucocorticoid receptors

The mode of action of corticosteroids commences following their diffusion across the cell membrane into the cytosol where they bind with glucocorticoid receptors (GR). Having done this, the activated GRs translocate into the nucleus of the cell to generate their effects on gene expression described above[82]. GRs bind in pairs to glucocorticoid response elements (GRE) in the promoter region of genes that are steroid responsive, with this interaction then switching on (or off) gene transcription. The main anti-inflammatory interactions are to switch off the multiple activated inflammatory genes that encode for chemokines, cytokines and adhesion molecules that have been switched on by pro-inflammatory transcription factors e.g. nuclear factor-κB (NF-κB), which activate downstream inflammatory genes, ultimately resulting in acetylation of core histones that facilitate the gene transcription. Activated-GRs reduce this histone acetylation through inhibiting NF-κB interacting with its co-activators, as well as through recruiting histone deacetylase-2 (HDAC2) to the activated inflammatory-gene complex. This then results in suppression of all activated inflammatory genes within the nucleus. Corticosteroid resistance, such as is seen in severe asthma or smoking asthmatics, is thought related to both a reduction in HDAC2 activity and expression[83]. Furthermore, reduction in the translocation of GRs via p38 MAP kinase phosphorylation is also thought to be important in corticosteroid resistance[84].

Effects on beta-2 adrenoceptors

Beta-2 adrenoceptors (B2ADRs) are one of a family of G-protein coupled receptors located on the cell membranes of smooth muscle cells in the airway. They are

primarily activated by the body's circulating catecholamines (e.g. adrenaline), or for the purposes of asthma treatment, this activation occurs via more selective synthetic substitutes – beta-2 agonists (i.e. SABAs and LABAs) - for relaxing airway smooth muscle and relieving airway constriction i.e. bronchodilation. However, in addition to the primary function of the B2ADR, there is a complex synergy between these and GRs - each benefitting the other when activated simultaneously[85]. We have already seen that gene transcription is switched on or off by activated GRs, and in fact one of these targets is the gene encoding for the B2ADR itself. Gene transcription for the B2ADR is increased by corticosteroids, resulting in greater expression of these cell surface receptors[86,87]. Furthermore, corticosteroids prevent, and indeed reverse, the down-regulation of B2ADRs that can be seen with chronic beta-2 agonist dosing[88-90]. Corticosteroids also enhance the coupling of B2ADRs to G-proteins to improve their effects, as well as protecting against uncoupling from exposure to inflammatory mediators[91,92]. Pointedly, corticosteroids have the ability to increase retention of LABA molecules on the airway surface[93], thus enhancing their clinical effect further. On the other side of the coin, beta-2 agonists themselves can reciprocally enhance the effects of the GR[80]. This process has been shown to improve the anti-inflammatory effects of corticosteroids at the nuclear level[80]. Moreover, studies have shown that the presence of both corticosteroid and beta-2 agonist moieties delivered at the same time to the airway has a synergistic effect on GR nuclear receptor translocation[94] and transcription[95], thus enhancing any anti-inflammatory effects further. This is one of the proposed reasons for the steroid-sparing effect of LABA therapy seen when added to ICS[96].

Pharmacokinetics and pharmacodynamics of different inhaled corticosteroids

Having considered the pharmacological effects of ICS as a whole, there is further complexity regarding the variety of different types of currently available ICS. (There is an even further level of granularity when also considering different delivery devices, but these are beyond the scope of this thesis so will not be mentioned in detail). The ideal pharmacological profile of an inhaled corticosteroid encompasses both efficacy and safety properties[97]. The properties of ICS considered advantageous for improved efficacy include: small particle size (increasing the fraction of dose delivered to the lung – particularly with particle sizes with a mass median aerodynamic diameter [MMAD] <2μm), high GR binding affinity, long pulmonary residence time and lipophilicity of the drug (all of which increase and prolong the ICS efficacy i.e. potency). Properties considered advantageous for an improved safety profile include: 'on-site' activation in the lung (reducing oropharyngeal effects), low oropharyngeal exposure, negligible oral bioavailability (usually achieved as a result of high first pass metabolism through the liver for ingested ICS), high systemic protein binding (reducing systemic adverse effects) and rapid systemic clearance.

Currently available ICS for asthma (British National Formulary, March 2015) include: fluticasone propionate (FP), fluticasone furoate (FF), budesonide (Bud), beclometasone dipropionate (BDP), ciclesonide (CIC), and mometasone furoate (MF). FP is available as both a dry powder inhaler (DPI) and hydro-fluoroalkane (HFA) suspension delivered via pressurised metered dose inhaler (pMDI). HFA has replaced chlorofluorocarbons (CFCs) as the propellants in inhalers for legally imposed environmental reasons. FF is a very new ICS that is given only in combination with a LABA for asthma as a DPI. Bud is delivered as a DPI in the UK.

(HFA)-BDP is delivered in a solution pMDI both singly and in combination with LABA – the solution formulation delivers a greater fine particle fraction to the lungs than suspension formulations. I have summarised the pharmacokinetic and pharmacodynamic properties of these currently available ICS in Table 1. Each one displays quite a number of the desired attributes for an 'ideal ICS', however; none of them present the 'full house' of qualities. Of note FP, FF, and CIC all display high levels of lipophilicity, improving pulmonary residence time and efficacy. BDP actually has a higher lipophilicity than FP, however, its active component beclometasone-17-monopropionate (B-17-MP), presented following conversion by esterases in the airway, has much lower lipophilicity than FP. CIC is the other ICS that is converted 'on-site' in the lungs to its active moiety desisobutyryl-ciclesonide (des-CIC), and to a greater degree than BDP. The highest receptor binding affinity, and long pulmonary residency of FF make this a once daily medication, whereas the remaining majority requires twice-daily administration (excluding CIC).

Table 1. Comparison of pharmacodynamic and pharmacokinetic properties of inhaled corticosteroids.

Inhaled Corticosteroid	Receptor Binding Affinity (RRA)	MMAD Particle Size (µm)	Lung Deposition (%)	Oropharyngeal Deposition (%)	Inhaled / Oral Bioavailability (%)		Protein Binding (%)	Lung Clearance (L/hr)	Terminal Elimination Half-life (hr)	Approx. Relative Doses* (µg)
FP (DPI)	1800	6.5	12-13	72-78	20 / <1		90	69	11	250
FP (HFA suspension)		2.4-3.2								
FF (DPI)	3000	4.0	No data	No data	15.2 / <1		99	Very long pulmonary residency	17-24	100
Bud (DPI)	935	2.5	20	62	15-30 / 11		88	84	2.3	400
BDP (HFA solution)	53	0.9	53	29	50-60	15	87	230	2.8	200
B-17-MP	1345					26		120		
CIC (HFA solution)	12	1.1-2.1	52	30-40	50 / <1		99	152	0.7	200
Des-CIC	1200			-				396	6.5	
MF (DPI)	2200	2.2	10-20	80	11 / <1		99	54	5.8	400

FP=Fluticasone propionate, FF=Fluticasone Furoate, Bud=Budesonide, BDP=Beclometasone dipropionate, B-17-MP=Beclometasone 17 monopropionate (active moiety of BDP), CIC=Ciclesonide, Des-CIC=Des-ciclesonide (active moiety of CIC), MF=Mometasone Furoate, DPI=Dry powder inhaler, HFA=Hydrofluoroalkane propellant (replaced chlorofluorocarbon), RRA=Relative receptor affinity (glucocorticoid receptor affinity relative to dexamethasone - denoted as having affinity of 100), MMAD=Mass median aerodynamic diameter (measured using Andersen cascade impactor). *Relative doses are approximate and based on an HFA-BDP equivalent dose of 200µg (The CFC-BDP equivalent dose would have been 400µg, but this is no longer relevant). References[97-110].

Adverse effects

ICS generally have a very good therapeutic index when it comes to adverse effects, particularly when used at low to moderate doses, as is the case in most asthmatics. The two main local side effects are that of dysphonia (hoarseness) and oral candidiasis. Dysphonia has been described in >50% of certain patient cohorts using a pMDI to deliver their ICS, but resolved on removal of treatment[111]. Oral candidiasis can also be a problem due to local delivery of a high proportion of the delivered ICS dose depositing on the oropharynx, rather than making a successful journey into the lung. However, this tends to be a problem mainly in the elderly, after oral corticosteroid use, or using one's ICS more than twice a day[112]. Furthermore, candidiasis (but not dysphonia) can be prevented by the use of a spacer or mouth rinsing[112]. There is no suggestion of an increased risk of pneumonia due to ICS use in asthma, as has been suggested in chronic obstructive pulmonary disease (COPD)[113].

When it comes to systemic adverse effects of ICS, they are certainly not as marked as can be seen with oral corticosteroid therapy. Indeed, whilst it may be possible to observe a measureable biochemical abnormality with ICS therapy, it is unclear how well this translates into a clinically important problem. However, once ICS dosing becomes very high, of the order of more than 1500µg/day (or 750µg/day for FP)[49] then systemic adverse effects can be seen. The main issue relates to suppression of the hypothalamic-pituitary-adrenal (HPA) axis by reducing corticotrophin production from the anterior pituitary gland leading to a reduction in cortisol secretion from the adrenal glands. This leads to the issue whereby on sudden discontinuation of ICS, patients may suffer an 'adrenal crisis' whereby the adrenal glands can no longer produce sufficient cortisol at times of stress – which is a potentially life-threatening

condition. However, overall, studies have been inconsistent in confirming this problem. There is some evidence that high doses of ICS can lead to posterior sub-capsular cataracts[49,114] as well as weaker evidence for the emergence of ocular hypertension and glaucoma[115]. Additionally bruising and thinning of the skin is seen with higher doses of ICS, which is again more apparent in the elderly[116]. In addition to adrenal suppression, another subject of intense debate and study with regard to systemic adverse effects from ICS therapy is that of bone demineralisation, osteoporosis, and thus increased fracture risk – where this is already a problem with regular oral corticosteroids causing rapid reductions in bone formation markers such as osteocalcin[117,118]. Studies on whether or not ICS deleteriously affects bone turnover are conflicting[119-121]. The impairment of growth in childhood by ICS is also an area of debate and controversy in this regard, but beyond the scope of this thesis as all subjects presented here have reached adulthood already.

Whether or not there are significant adverse effects with ICS therapy, the important issue is to ensure that asthmatic patients are treated with the lowest dose of ICS that controls their disease, as the potential therapeutic benefits of ICS certainly outweigh the risks of adverse effects for the large majority of patients.

MEASURING DOSE-RESPONSE TO ICS IN PERSISTENT ASTHMA

Lung function / Airway calibre

Measures of pulmonary function include peak expiratory flow rate (PEFR), Spirometry and Impulse Oscillometry (IOS). PEFR and Spirometry (in particular FEV_1) are well established as outcomes of treatment response in asthma, whereas IOS is only just emerging as such with the rejuvenated interest in the role of the peripheral 'small' airways (<2mm) in asthma[122].

PEFR and spirometry are readily available and easy to perform in both primary and secondary care, and even as domiciliary measurements. Spirometry is also very reproducible, making it ideal for serial measurements[123]. Importantly, it has been shown that the odds of having asthma symptoms increase with decline in FEV_1[124], albeit the overall predictive value of FEV_1 for this reason has been suggested to be only 70%[125]. It is also important to know the minimum change in any outcome measure that relates to a perceptible change in patient wellbeing – otherwise known as the minimal clinically important difference (MCID) – as a gauge of the clinical relevance of any measured difference. Santanello and colleagues suggest that PEFR has a MCID of around 19L/min, where for FEV_1 a change of 0.23L is thought clinically relevant[126]. While these degrees of improvement are readily surpassed when steroid-naïve asthmatics are commenced on ICS even at low dose, the dose response for lung function beyond low dose ICS is remarkably flat. This is neatly demonstrated in two Cochrane reviews both examining dose responses to FP, with the first comparing increasing doses of FP to placebo[44] that shows large improvements in FEV_1 for all doses versus placebo. Whereas the second study compares different

doses of FP[127] showing more meagre improvements between doses that don't meet the putative MCID for FEV_1. An earlier meta-analysis, again using FP, also showed this plateauing of the dose-response between 100-200µg for both PEFR and FEV_1, with a peak effect at around 500µg of FP. Indeed 600µg of FP was roughly equivalent to a change of 0.71L in FEV_1 (MCID=0.23L) and 50L/min in PEFR (MCID=19L/min) over placebo. This early plateauing of the dose-response curve for ICS versus lung function has been repeated in other studies[128], but there is the caveat of a significant inter-individual variability with regard to these responses. Importantly, there does not seem to be a significant difference between different ICS with regard to the point at which this plateau in dose-response for lung function occurs, as demonstrated in another Cochrane review comparing FP, Bud and BDP[129].

Impulse oscillometry (IOS) is a non-effort dependent measure of airways resistance performed during relaxed tidal breathing (PEFR and Spirometry are both effort-dependent), and can provide differential measures of total airway resistance at 5Hz (R5), central large airway resistance at 20Hz (R20) and derived measures of peripheral small airway resistance (R5-R20 and Ax). There are no studies examining the dose-response to ICS in asthma with IOS outcomes. However, in a study comparing HFA-BDP with CFC-BDP (i.e. extra-fine particle ICS versus standard size particle ICS), IOS indices improved with both formulations in line with improvements in FEV_1[130]. Pointedly, however, for HFA-BDP there was a significantly greater improvement in the peripheral 'small' airway measurement of R5-R20 than seen for CFC-BDP. There are no data on what any MCID might be for IOS measurements at the present time.

Airway hyper-responsiveness (AHR)

AHR is defined as an abnormal response of the airways to a stimulus that results in excessive airway constriction in asthmatics, where the same stimulus would have little or no effect on the airways of a healthy individual. AHR is therefore well recognised as a hallmark of the asthmatic process. Yet AHR is still not fully understood in terms of its causal pathophysiological mechanisms. However, we do know it is broadly affected by underlying (allergic) airway inflammation[131-134], hyper-contractility of airway smooth muscle[135], and airway closure itself[136] (this last particularly with regard to peripheral small airway closure[137]). Measuring AHR is useful not only for aiding asthma diagnosis, when spirometry may be normal, but also in the classification of its severity[20,138] and in tailoring ongoing management[6,13]. Furthermore, AHR has been shown to predict: ongoing decline in lung function[139], the risk of developing asthma[140], severity of asthma symptoms[141], risk of exacerbation[46] and an increased level of treatment[142]. Despite the clear utility of non-specific measures of AHR, individual patients can respond very differently to the variety of bronchial challenge tests that are available, presumably due to heterogeneity of the underlying mechanisms driving their individual (presence or absence) of AHR[143]. Moreover, even with any given test of AHR, there is significant within-individual variability of the test result itself, making determination of any MCID difficult. Indeed this has been proposed to range between 1-3 doubling dilutions/doubling doses of the challenge agent used to cause the same fall in FEV_1[144].

There are two main categories of bronchial challenge testing – direct and indirect. 'Direct' bronchial challenge tests include methacholine and histamine that act directly on the airway smooth muscle to cause bronchoconstriction. Challenge tests termed

'indirect' cause bronchoconstriction through invoking the underlying allergic/inflammatory processes thus indirectly releasing inflammatory mediators that then trigger the airway smooth muscle to contract. These indirect challenge agents include: adenosine monophosphate (AMP), mannitol, allergen, exercise and eucapnic voluntary hyperventilation (EVH). Of these, mannitol seems to have emerged as the one with greatest clinical utility in terms of ease of practical implementation and good relationship to other challenge agents such as methacholine[131]. Furthermore, because of their mechanism, indirect challenges have been shown to bear a greater relationship to airway inflammation in asthma than do the direct challenge tests[131,132].

There is a multitude of evidence showing that AHR responds favourably to ICS. In one study examining high doses of Bud in severe asthmatics, even the lower dose of 1200μg/day caused a 3 doubling dose improvement, whilst the higher 3200μg dose achieved an only slightly higher improvement of 3.2 doubling doses (of histamine)[145]. However, patients receiving the higher dose for the first 16 weeks of the study were almost 4 times as likely to normalise their AHR. In a meta-analysis by Currie and colleagues comparing the dose-responses of a variety of ICS on AHR[146], they found that high dose ICS (≥1000μg/day) caused a significantly greater degree of improvement in AHR with a 2.16 doubling shift over placebo as compared to low/moderate ICS dosing (<1000g/day) at a 1.25 doubling shift over placebo. Additionally, several ICS intervention trials[6,13] have shown that by titrating (up) ICS dose with the target of improving AHR, compared to just targeting symptoms, then this results in a reduction in the number of mild exacerbations, suggesting that overall asthma control might be better using this method of tailored ICS treatment. This is

certainly an area requiring greater study to try to pin down how best to personalise asthma care in relation to the variety of underlying mechanisms that cause AHR[135].

Airway inflammation

Airway inflammation is the other main hallmark of the asthmatic disease process, along with AHR. It has been identified histologically[147,148] and even in 'mild' asthmatics[39] as discussed previously. Clearly endobronchial biopsies are not going to be widely useful as a measure of airway inflammation, due to the invasive nature and risks associated with this procedure. However, surrogate markers of airway inflammation have been identified that have proven useful in the identification, monitoring and management of asthma. The two main biomarkers that I will discuss here in relation to ICS therapy are sputum eosinophilia and exhaled nitric oxide (FeNO).

The eosinophil is one of the key cells produced as part of any allergic TH2-driven inflammatory pathway. They are easily identified in higher numbers than normal in both sputum and blood in asthmatic patients[149]. To complicate matters slightly, eosinophilic asthma is considered one of the main phenotypes of asthma, as compared to non-eosinophilic asthma[150]. Sputum eosinophils have been shown to be clinically relevant in asthma for several reasons. Firstly they predict loss of asthma control[151] and increased exacerbation[152] rate upon withdrawal of ICS therapy. Pointedly, it has been shown that adjusting asthma therapy on the basis of targeting a reduction in sputum eosinophil levels in moderate to severe asthmatics, significant reductions in the number of severe exacerbations experienced in the sputum eosinophil group were

achieved – 35 versus 109 compared to those whose treatment was tailored in a traditional method[14]. Surprisingly, the mean doses of inhaled and oral steroid used to achieve this landmark improvement was not higher in the sputum eosinophil group compared to standard therapy (therefore, not a dose-response phenomenon) – presumably because higher treatment doses were targeted at the right time (when inflammatory levels were highest), thus evening out the dosing over the 12-month period. This further shows that personalising asthma therapy is both appropriate and effective. A dose response to ICS for sputum eosinophils in asthma has however been shown for MF in a study comparing 50µg twice daily versus 400µg twice daily on suppression of late-phase response to allergen challenge (an indirect challenge)[153], albeit the difference in effect between the lower and higher dose of MF was not large, i.e. alluding to an early plateau effect.

The other non-invasive method of measuring airway inflammation in asthma is with fractional exhaled nitric oxide (FeNO) levels[22]. Previous studies have confirmed that FeNO levels are higher in asthmatic patients than in healthy controls[154,155], albeit there is still a degree of within-subject variability and between-subject overlap[128]. FeNO has also been shown to correlate with both eosinophil levels[156] and AHR[132,157]. Furthermore, higher FeNO levels in asthma are associated with a higher risk of future exacerbation and can predict those who are likely to respond to ICS[155]. Indeed, more recently it has been shown to be a helpful measure of compliance to ICS therapy[158]. FeNO can now be easily measured on a hand-held device (still 2 hands rather than one!), with this increased portability useful for domiciliary or clinic measurement of FeNO levels. In a similar vein to that of the Lancet study by Green *et al* targeting sputum eosinophils, Smith *et al* used FeNO measurements to guide treatment in

asthma comparing ICS dose adjustment according to either FeNO levels or conventional guidelines[15]. However, they found that overall ICS doses could be significantly reduced while keeping the same level of asthma control i.e. no change in the exacerbation rate. Furthermore, levels of sputum eosinophils were not different between groups. I believe this tells us two things: firstly that FeNO responds very well to lower doses of ICS, and secondly that FeNO does not correlate well with either symptoms or lung function, which are the standards currently used in assessing asthma control. In terms of a dose-response relationship of ICS to FeNO levels, Silkoff *et al* showed that dose separation of three doses of BDP was better when measured by FeNO as compared to no separation seen with FEV_1 or methacholine AHR in originally steroid-naïve asthmatics – however this separation was only seen between the lowest (100µg/day) and highest (800µg/day) doses of BDP, again showing dramatic improvement on low dose ICS, but less so with higher dosing[159]. Furthermore they found that the reduction of FeNO was highly reproducible on stopping and then restarting the ICS. This dose response has also been shown in another study, again using BDP[160].

Smith *et al* have shown that both sputum eosinophilia and FeNO are better for diagnosing asthma than spirometry or symptoms, with negative predictive values at 92% for both[161]. Indeed, a recent paper by Cowan *et al* suggests using a panel of inflammatory biomarkers to enable better prediction of ICS responsiveness in asthma, particularly as the presence of high levels of sputum eosinophils and FeNO do not always overlap[162].

Symptoms, reliever use and asthma control

The outcome measures of symptoms, reliever (SABA) use and asthma control (the definition of which varies widely between studies) are all necessarily inter-related. Despite being most relevant to individual asthma patients' daily lives (where FEV_1 is not), they are most often utilised as secondary outcomes in studies of asthma rather than as *a priori* primary outcomes. Nevertheless, there is still good evidence of a dose response to ICS for these outcomes when one delves deeper into a variety of studies examining the utility of ICS on other asthma outcomes. In one study of long-term treatment with Bud 400µg/day for 1 year versus placebo in non-steroid dependent asthmatics[40], there were significant improvements seen in both asthma symptoms (even though only mild to begin with) and rescue bronchodilator use over the course of the year. Indeed symptoms continued to improve out as far as 9 months then plateaued, whereas the improvements in bronchodilator use plateaued at 3 months – with no change with placebo for either outcome. Asthma symptom scores also improved in a meta-analysis of the dose-response to several different ICS, albeit again with most improvement seen with the lowest doses of ICS[41]. Furthermore, in studies that showed the advantage of ICS/LABA combination over increasing ICS dose alone, the ICS alone arm still showed significant improvements in symptoms and beta-2 agonist use[42,63,129].

Measures of symptoms, health-related quality of life (HRQoL) and asthma control are problematic to formulate, due to the heterogeneity of presentation, severity, variability and time course of this disease. They need to be wide-ranging to encompass all the possible variations; yet specific enough to asthma, in order for any meaningful clinical change to be measureable upon commencement or change in asthma therapy.

Moreover, patients' interpretation of their own control may underestimate the degree of disability they may have[30], without using a formal validated measure. A number of symptom diaries have been validated in this regard. For example, Santanello and colleagues[163] validated 2 diary scales for daytime and nocturnal symptoms in a cohort of 346 adult asthmatics in response to trials examining a leukotriene biosynthesis inhibitor, encompassing activity during the day, showing good test-retest reliability (0.69-0.87) and strong correlations to other outcome measures including FEV_1 and reliever usage. However, many measures of symptoms and other diaries in clinical trials are uncontrolled and not well validated, so their interpretation should be guarded.

The next level beyond pure symptom measurement is that of health-related quality of life (HRQoL). There are many general measures of HRQoL, but again for the purposes of greater specificity to asthma and its treatment, questionnaires have been developed that more closely relate to these. The most widely used, and well validated of these is the Juniper Asthma Quality of Life Questionnaire (AQLQ)[164,165]. This provides an overall score (32 questions and 4 domains), as well as the ability to tease out responses to individual domain components comprising symptoms, activities, emotions and environment. Importantly, it has been shown to have a reliable minimal clinically important difference of 0.5[166], which is useful for interpreting interventions in asthma trials, and clinical response to asthma treatment. It has also been shown to correlate well with the Asthma Control Questionnaire (ACQ)[33]. There is a shorter version of AQLQ, namely the mini-AQLQ[35] which is probably more widely used due to greater ease of administration, but it is not quite as powerful a tool as its bigger brother.

There has been a palpable move away from just using symptoms, quality of life or indeed simple spirometry to guide treatment in the literature; with the recognition that measuring overall asthma control as being more important. We do not yet have a tool that encompasses all aspects of the asthmatic disease process, namely: symptoms, variable airflow obstruction, AHR and airway inflammation. Nevertheless, the reason this approach is more important, is that measures of overall asthma control both more consistently follow meaningful improvements with asthma treatments as well as, perhaps even more importantly, predicting loss of control and future risk of adverse outcomes. In the GOAL study, Bateman et al. were able to show that by religiously tailoring asthma treatment to achieve 'well-controlled' asthma using a composite guideline-based measure over and above single component improvement, that greater achievements in quality of life could be gained[32]. Furthermore, current measured levels of asthma control can, not only predict loss of control[46], but also future risk of exacerbations[28,29]. Once again the most commonly used of these composite measures of asthma control are the Asthma Control Questionnaire (ACQ)[33] and the Asthma Control Test (ACT)[167] (Appendix II). The ACQ comprises patient recall of the previous 7 days for breathlessness, nocturnal wakening, symptoms on waking, activity limitation, wheeze, frequency of SABA use, and pre-bronchodilator FEV_1 %predicted. There is a 7-point scale for each with no weighting applied, with higher scores indicating worse control. Once again, it has a minimal clinically important difference of 0.5, and shorter versions are available with similar clinical utility to the full version, which are simpler to administer, for example, in primary care[168]. Over and above the MCID of 0.5, useful cut-off points have been established for the ACQ, where a score of ≤ 0.75 equates to 'well-controlled' asthma using the GOAL classification, and a score ≥ 1.5 determines 'not well-controlled' asthma[34]. Indeed, an

ACQ≤0.75 has a negative predictive value of 0.85, where an ACQ≥1.5 has a positive predictive value of 0.88, to correctly identify both 'well controlled' and 'not well controlled' asthma respectively. For the ACT score, it also relates well to the GINA defined classification of asthma control with an area under the receiver operating curve of 0.84[169]. Despite the importance of these composite measures of control, few studies have used them as a primary outcome measure[29].

I have summarised advantages and disadvantages of these various measures in Table 2 regarding their utility in asthma and detection of ICS dose response.

Table 2. Properties of asthma outcomes on ICS dose response.

Measurement	Advantages	Disadvantages
Airway Calibre (FEV_1, PEFR)	Simple to use. Reproducible. Recognised MCID. Intra-individual repeatability.	Insensitive in mild asthma. Early plateau in ICS response. Effort dependent.
Small airways (Impulse oscillometry)	Non-effort dependent. More sensitive for peripheral airways than spirometry.	Not widely available. Limited experience outside research institutions. Large coefficient of variation compared to spirometry.
Airway Hyper-responsiveness	Aids positive identification of asthma diagnosis. Predicts future risk of loss of control / exacerbations. Good dose response relationship to ICS.	Cumbersome to administer (perhaps with the exception of mannitol challenge). Not widely available.
Inflammation (Sputum Eos)	Predicts loss of control. Targeted therapy improves outcomes.	Difficult to collect good samples. Cumbersome processing of samples.
Inflammation (FeNO)	Easy to measure with handheld device. Predicts ICS response and loss of control. Can monitor ICS compliance. Improves diagnosis of asthma (excellent negative predictive value).	Not widely available. Expensive technology. Intra-individual variability.
Symptoms & Reliever use	Simple to measure. Relevant to patient.	Wide variety of symptom scores / diaries, not all well validated. Early plateau in ICS dose response.
Asthma control & Quality of life	Well-validated tools available. Detects clinical change to treatment (validated MCIDs). Easy to administer. Predicts future loss of control / exacerbations.	Does not measure airway inflammation or AHR.

Effects of extrinsic factors

In addition to measurement of lung function, symptoms, AHR and inflammation in asthma with regard to ICS dose response; external factors can also play a part in an individual's responsiveness to ICS therapy. Such extrinsic factors include, but are not limited to: smoking; obesity; allergic rhinitis; and gastro-oesophageal reflux disease (GORD).

Smoking asthmatics have worse symptoms[170], and are more likely to be admitted to hospital with an exacerbation than non-smoking asthmatics[171]. Furthermore smoking cessation improves asthma control in these individuals but the rates of smoking cessation are low[172]. It has also previously been shown that smoking asthmatics are more resistant to the effects of ICS[173,174] than their non-smoking counterparts. Proposed molecular mechanisms for this relative steroid resistance in asthmatics who smoke include: non-eosinophilic airway inflammation, impaired glucocorticoid receptor function with switch over from alpha to beta-glucocorticoid receptor subtypes, along with a reduction in histone deacetylase activity and expression which switches off activated inflammatory genes[83,172]. The obvious solution is to stop smoking, but this proves difficult for many individuals. Other approaches that may help include the addition of a LABA to ICS[175], or indeed the use of leukotriene receptor antagonists[176].

Obesity is an increasing problem, particularly in relatively affluent countries, and so asthmatics are not excluded from this phenomenon. Indeed, asthma itself has a higher prevalence in westernized countries, therefore, increasing the numbers of obese asthmatics further[177]. This is important because obese individuals have been shown to

have a higher prevalence of asthma than non-obese asthmatics[178]. Furthermore it is an independent predictor of worsening asthma control[179]. One study showed that an overweight group of asthmatics had fewer asthma control days than their normal weight and otherwise matched counterparts[180]. In vitro responses to glucocorticoids in overweight asthmatics revealed that dexamethasone response markers were blunted, including tumour necrosis factor levels, theorizing reduced clinical efficacy in this group[181]. Additionally, bariatric surgery to aid weight loss resulted in improved AHR in obese asthmatics, adding more weight to the argument that obesity contributes to the symptoms and severity of asthma[182]. There have been no ICS dose response studies in this regard, until reported later in this thesis.

Allergic rhinitis and its association with asthma is a very large subject by itself, with international (ARIA) guidelines[183] dedicated to its management. It shares the underlying TH2 driven pathophysiology of allergic asthma, and as such the concept of the 'unified allergic airway' was coined. Whether it is a distinct phenotype of disease or simply comorbidity can be debated, however, it is clear that in individuals who suffer both conditions concurrently, there is greater impairment of quality of life over those with either single disease alone. Furthermore, Corren et al. showed that in patients with both asthma and allergic rhinitis, the odds ratio for hospitalisation due to asthma was significantly reduced when their allergic rhinitis was treated with a nasal steroid, OR 0.56 (95% confidence intervals 0.42-0.76)[184].

GORD is certainly prevalent in patients with asthma, and oesophageal acid may alter airway hyper-responsiveness[185]. In a meta-analysis of GORD medical therapy in 326 patients with both asthma and GORD[186], it was reported that anti-reflux therapy

improved not only asthma symptoms, but also medication use (including ICS) by 62%, additionally improving evening PEFR. However another systematic review reported that anti-reflux therapy does not consistently improve asthma control in patients with both conditions[187]. There have been no ICS dose response studies in this regard, but it is possible that in patients with both asthma and GORD, their ICS dose response might be attenuated.

MOVING TOWARDS PERSONALISED ASTHMA TREATMENT (PHENOTYPES)

A striking observation about the most recent iteration of the BTS asthma guidelines[10] is that the overwhelming majority of the document is a management recommendation for 'all-comers' with asthma, even though it is recognised that asthma is a heterogeneous disease. This is understandable to a degree, given the majority of patients will respond favourably to low to moderate doses of ICS alone. However, there is growing interest in identifying and studying specific asthma phenotypes[188], which may help us understand more about the pathophysiology of this heterogeneous disease; with the aim of leading on to the discovery and implementation of novel, individualised asthma therapies. A phenotype is the presentation of outward characteristics that result from the expression of an organism's genes as well as the influence of environmental factors and the interactions between the two. Furthermore, an endotype is a subtype of a condition, which is defined by a distinct functional or pathobiological mechanism[189,190]. It is difficult to be dogmatic about the difference between phenotypes and endotypes, as there will necessarily be a degree of overlap, but even mechanistic traits need to be measured by some means.

The earliest form of asthma phenotyping came with the recognition of two distinct groups – namely 'Allergic' and 'Non-allergic' asthma. Over the years other phenotypic characteristics have been used to help explain the diversity of asthmatic patients. These include: childhood asthma; atopy; reversible airflow obstruction; intermittent vs. persistent symptoms; severity of airflow obstruction; corticosteroid responsiveness and others. However, single characteristic phenotypes can be subject to bias. To bring phenotypes more into focus it has been important to consider

endotypes of asthma in this regard. To progress to this level of clarity has required complex statistical cluster analyses, molecular biomarkers and other genetic approaches to cluster asthmatic patients into easily identifiable groups – with the ultimate aim of personalizing treatment for each individual.

Phenotypic cluster analyses[191-193] have found a number of common, reproducible groups between studies including: benign or mild untreated asthma; early-onset atopic/allergic asthma; active, treated, atopic early-onset; female, symptom predominant; obese, non-eosinophilic; late onset, associated with nasal polyps; very severe, systemic corticosteroid dependent. Importantly these have been correlated with treatment response and asthma control. In the Haldar clusters[191], treatment according to inflammation led to a reduction in exacerbations for the 'inflammation predominant' cluster, and reduced steroid dose in the 'symptom predominant' cluster. In the Siroux clusters[192] there were strong associations with AQLQ for all clusters as well as blood eosinophilia. Similarly, severe asthma endotypes have been identified[194]: early-onset allergic; persistent eosinophilic; obese female; neutrophilic; allergic bronchopulmonary aspergillosis (ABPA).

Biomarkers, predominantly linked to the allergic TH2 pathway, have been identified as part of pheno/endotypes: eosinophils (sputum and blood); neutrophils (sputum); IgE; FeNO; Interleukins (5,13,17); serum periostin (drives IL-13 pathway). As we have already discussed, eosinophils and FeNO predict corticosteroid responsiveness. The Anti-IgE therapy Omalizumab is reasonably successful in refractory atopic asthma, in selected individuals. Total-IgE is elevated in allergic clusters of patients, but omalizumab is expensive and it remains difficult to predict responders to this

medication[195]. Novel anti-IL5 (mepolizumab) and anti-IL13 (lebrikizumab) agents are showing significant promise for personalizing treatment. Mepolizumab has been used in refractory eosinophilic asthma[196]. It significantly reduced exacerbations and eosinophil counts, as well as improving AQLQ scores by 0.55 (MCID is 0.5) from baseline. There was no change, however, in symptoms, FEV_1 or AHR. IL-13 drives the allergic TH2 pathway and levels of circulating IgE. This has been targeted using Lebrikizumab[197], where the authors found that treatment response to this agent could be differentiated further using levels of serum periostin, i.e., a greater response was seen (in pulmonary function) in patients with higher baseline periostin levels.

Endotyping by genetic approaches has also yielded results. The pharmacogenetics at the Beta-2 adrenoceptor (Arg16Gly) predicts response to SABAs and LABAs as well as asthma control[198-201]. Additionally gene clustering has confirmed 'TH2-high' and 'TH2-low' endotypes in terms of their display of allergic features[202]. More recent work with sputum gene clusters shows these can predict the predominant cell type, as well as inhaled corticosteroid response[203]. 'Stand alone' endotypes include aspirin sensitive asthma, which has demonstrated the utility of leukotriene receptor antagonists here[204]. Allergic bronchopulmonary aspergillosis (ABPA) is another clearly defined asthma endotype with specific features and a tailored treatment regimen[205].

The role of co-morbid conditions impacting on asthma cannot be ignored, but do not necessarily fit into a particular phenotype or endotype. However, I have already discussed these in the previous section. Future areas of study in determining specific asthma phenotypes and endotypes may come through clearly defining the role of the

small airways in asthma. There are also new monoclonal anti-bodies, for example, anti-IL17 that hold some promise.

In summary, there has been significant progress in recent years in identifying useful phenotypes and endotypes of asthma. These have predominantly been within the umbrella of allergic asthma. They have helped to individualise treatment options for narrow groups of asthmatic patients. However, more work in clarifying these clusters of patients is required, as well as exploring novel areas of pathophysiology. Additionally, the non-allergic group of asthmatic patients has limited treatment options, so expansion of similar work in this group is urgently needed.

SUMMARY AND THESIS OBJECTIVES

Asthma is a common, complex, and heterogeneous disease of the airways. Despite the great utility of inhaled corticosteroids as an effective controller treatment for asthma in the last half-century or so, there is still significant ongoing morbidity and mortality that is theoretically avoidable. We still have difficulty achieving accurate asthma diagnoses, predicting response to asthma therapies, finding the correct therapy for an individual, and perhaps most importantly, identifying those patients who are at greatest risk of future exacerbation and ongoing disease morbidity that require further pre-emptive manipulation of their asthma treatment.

In this thesis, the broad theme of examination is that of inhaled corticosteroid dose response with incremental ICS dosing on a variety of clinical outcome measures, including those that are less routinely measured, i.e., airway hyperresponsiveness, airway inflammation and asthma control. The overall aim, therefore, is to generate evidence from these studies that will add to the clinical decision-making process regarding individualised ICS therapy for persistent asthmatics.

The first two studies will examine the dose response of ICS on two common and major specific factors that influence asthma. One study examines an intrinsic factor, with the second study an extrinsic factor. The intrinsic factor is airway inflammation as measured by daily domiciliary fractional exhaled nitric oxide (FeNO). The extrinsic factor is the common co-morbidity of obesity. Clinical outcome measures of symptoms, pulmonary function, airway hyperresponsiveness (AHR) and inflammation will be observed to determine how best to follow and utilise the ICS dose response in these specific groups.

The third study in this thesis presents an examination of potential pharmacological manipulation of the ICS dose response, in order to investigate whether the inverse agonist propranolol (a non-selective beta-blocker) had any steroid-sparing properties in this regard. It had previously been shown that nadolol, another inverse agonist, improved airway hyperresponsiveness in steroid naïve asthmatics. We believed, however, that it was more clinically relevant to examine this question in asthmatic patients already receiving ICS, both for reasons of safety (with regard to beta-blocker administration in asthmatics) and with the knowledge that low dose ICS are an already safe and effective treatment in mild steroid naïve asthmatics.

The following two studies in this thesis address: how inflammatory outcomes relate to each other as well as to more routine outcomes in patients receiving ICS; in addition to an overall assessment of the ICS dose response characteristics across a broad range of both ICS moieties and outcome measures. The final study presented in this thesis examines how ICS at low versus higher doses affect sensitive markers of bone turnover; thus addressing one of the major potential systemic effects of higher ICS dosing over a 1-year period.

CHAPTER 2:

METHODS

There are detailed protocols described within each individual study presented in this thesis. However, there are a number of methods common to most studies that are therefore described here.

ASTHMATIC PARTICIPANT SELECTION

Participants with asthma were directly recruited from the database held in the Asthma and Allergy Research Group (now Scottish Centre for Respiratory Research) at the University of Dundee. This dataset includes all patients who have contacted the Asthma and Allergy Research Group in order to help with participation in clinical trials, and who provided their consent for their contact details to be kept securely. It is kept regularly updated by administrative staff within the research group. In addition to this recruitment database, patients were offered the opportunity to take part in clinical trials within the specialist asthma clinic at Ninewells Hospital and Medical School, Dundee. Finally, general advertisements to take part in clinical trials of asthma were made in the hospital, university and local press to aid further recruitment.

Patients were invited to attend for a screening visit based on their likely suitability for study entry based on their recorded data that included: age, onset of asthma symptoms, lung function, symptom scores, current treatment regimen, airway inflammation and airway hyperresponsiveness. Patients were in receipt of a written participant information sheet that provided details of specific study involvement and requirements, before attending for screening. All participants were given the

opportunity to ask questions and provided written informed consent in the presence of either a study investigator or research team member who had been delegated the task and had appropriate good clinical practice (GCP) training.

All patients included in these studies were aged between 18 and 65 years upon study entry. Furthermore, they all had a confirmed physician-based clinical diagnosis of asthma according to current guidelines[10]. Patients were required to go through a screening process for their safety prior to study entry that demonstrated a normal physical examination by a doctor, urinalysis, and screening blood tests comprising: full blood count, urea and electrolytes, liver function tests and random blood glucose. The details of the specific study entry criteria are provided within the individual study.

All study protocols, patient information sheets, informed consent forms and other study materials were scrutinized and approved by the East of Scotland Research Ethics Committee. Furthermore, for clinical trials of investigational medicinal products, further review and approval was gained from the Medicines and Healthcare products Regulatory Authority (MHRA) prior to study commencement.

AIRWAY MEASUREMENTS

Spirometry

Spirometry was performed in all studies following the recommended guidelines from the American Thoracic Society[123]. Patients were instructed to inhale fully to total lung capacity, and then forcibly exhale all the way out to residual volume through the mouthpiece attached to a mass-flow sensor. Measures of FEV_1, FVC and FEF_{25-75} could then be determined in absolute terms as well as compared to the normal population distribution as percentage of predicted (according to age, sex, height and ethnicity). All measures were performed in triplicate to within a 5% tolerance, with the highest achieved value recorded. Studies used the SuperSpiro (Micro Medical; Rochester, Kent, UK) spirometer. Withholding times for asthmatic therapies prior to screening spirometry were as follows: antihistamines, 7 days; theophyllines, 5 days; LTRAs, 5 days; LABAs, 2 days; Ipratropium bromide, 12 hours; salbutamol or bricanyl, 6 hours.

Domiciliary FEV_1 and peak expiratory flow rates were performed by subjects on a daily basis during selected studies. Measures were once again performed in triplicate both morning and evening using the electronic PiKO monitor (n-Spire Health, Longmont, Colorado, USA) according to the manufacturer's instructions[206]. Recorded values were stored on the electronic device, and then downloaded to custom computer software where they were checked for validity before being recorded for the subject.

Impulse oscillometry

Impulse oscillometry (IOS) measurements were performed according to published guidelines[207] and manufacturer's instructions. IOS is a non-effort dependent test of airway resistance and reactance performed during tidal breathing against a variety of sound waves of pre-specified amplitudes. Participants were required to hold both cheeks gently to prevent puffing and therefore shunting of the pressure wave. The impulses were applied over 30 seconds of tidal breathing. All measures were performed in triplicate with mean values calculated. The Masterscreen Impulse Oscillometer (Jaeger, Höchberg, Germany) was used where included as a study outcome measure.

Bronchial challenge tests

Direct bronchial challenge tests performed in these studies included both methacholine and histamine bronchial challenges. These are used to determine both the presence and severity of airway hyperresponsiveness (AHR) in asthma. These are termed 'direct' challenge agents because their mode of action is directly on airway smooth muscle receptors leading to bronchoconstriction. The provocative concentration of challenge agent that causes a ≥20% fall in FEV_1 (PC_{20}) is the measure used to determine the degree of AHR. A value ≤8mg/ml is considered evidence of an abnormal level of AHR to either challenge[20]. Severe AHR is deemed a PC_{20}≤1mg/ml.

Methacholine was made into increasing doubling dilution concentrations ranging between 0.03-32mg/ml. For Histamine this ranged between 0.3125-40mg/ml. Tayside Pharmaceuticals (Ninewells Hospital, Dundee) prepared these solutions using benzyl alcohol as the diluent. The tests were performed according to recommended guidelines with a validated computer assisted dosimetric protocol[20]. Patients were excluded from study participation if their FEV_1 was <60% predicted for safety reasons, i.e. to avoid life-threatening bronchoconstriction. Suitable patients then had an FEV_1 measurement performed using the diluent alone, where this was the baseline used to determine the eventual PC_{20}. Methacholine or histamine were subsequently administered in doubling cumulative doses at 5 min intervals until a 20% fall in FEV_1 was surpassed at a given dilution of challenge agent. The actual PC_{20} values were then calculated with the aid of a computer program that logarithmically interpolated this from the dose-response curve[208]. If a 20% fall in FEV_1 could not be achieved, then the PC_{20} value was capped at 64mg/ml for statistical analysis purposes for both challenge agents. Serial FEV_1 measurements were made using a SuperSpiro (Micro Medical; Rochester, Kent, UK) spirometer connected to the computerized dosimetry software.

Indirect bronchial challenge agents utilise and invoke the underlying inflammatory cascade in asthmatic airways to generate bronchoconstriction. We used mannitol bronchial challenge in these studies. Mannitol is an osmotic agent that dries out the airway epithelium that leads to release of inflammatory mediators that subsequently cause airway narrowing. Mannitol dry powder (Aridol, Pharmaxis, Sydney, Australia) bronchial challenge was performed as previously recommended[209]. Using the supplied dry powder inhaler device, patients serially inhaled doubling doses thus:

0, 5, 10, 20, 40, 80, 160, and 160mg of mannitol. FEV_1 was measured 60 seconds after each inhalation, with the highest value of three recorded. The test ended once a 20% fall in FEV_1 from the 0mg dose was surpassed, or when the maximum dose of 635mg had been given. The mannitol provocative dose to cause 10%, 15% and 20% falls in FEV_1 (PD_{10}, PD_{15}, PD_{20}) could then be calculated again using log-linear interpolation of the dose response curve.

Withholding times for asthmatic therapies prior to screening bronchial challenges were as follows: antihistamines, 7 days; theophyllines, 5 days; LTRAs, 5 days; LABAs, 2 days; Ipratropium bromide, 12 hours; salbutamol or bricanyl, 6 hours. Patients were either allowed to spontaneously recover their bronchoconstriction with serial recording of FEV_1 until within 5% of pre-challenge baseline, or they were given salbutamol 400µg immediately post-challenge, dependent on the study.

QUALITY OF LIFE AND ASTHMA CONTROL MEASURES

Diary cards

In studies with defined treatment periods, patients were asked to complete diary cards, recording twice-daily measurement of domiciliary FEV_1 (described above), rescue beta-2 agonist use and to assess their symptoms. Symptoms were based on a rating scale of 0-3, comprising: 0 – no symptoms, 1 – mild symptoms, 2 – significant symptoms, 3 – debilitating symptoms. These recordings were made during all run-in, treatment and washout periods.

Asthma Quality of Life Questionnaire

The Juniper Asthma Quality of Life Questionnaire (AQLQ)[164,165] provides an overall score (comprising 32 questions and 4 domains), as well as the ability to tease out responses to individual domain components pertaining to: symptoms; activities; emotions; and environment. There is a smaller version, also used, called the mini-AQLQ (comprising 15 questions with the same 4 domains). Each question is on a 7 point scale; the responses are then averaged for each of the four domains, which themselves are subsequently averaged to provide the final score. A score of 7 indicates no impairment, and anything <7 indicates increasing impairment. Importantly, it has been shown to have a reliable minimal clinically important difference of 0.5[166].

Asthma Control Questionnaire

The Asthma Control Questionnaire (ACQ)[33] comprises patient recall of the previous 7 days for: breathlessness; nocturnal wakening; symptoms on waking; activity limitation; wheeze; frequency of rescue beta-2 agonist use; and pre-bronchodilator FEV_1 as %predicted. There is a 7-point scale for each domain with higher scores indicating worsening control. Once again, it has a minimal clinically important difference of 0.5, and shorter versions are available with similar clinical utility to the full version, which are simpler to administer, for example, in primary care[168]. Over and above the MCID of 0.5, useful cut-off points have been established for the ACQ, where a score of ≤0.75 equates to 'well-controlled' asthma, and a score ≥1.5 determines 'not well-controlled' asthma[34].

FRACTIONAL EXHALED NITRIC OXIDE

FeNO was measured in the relevant studies using the portable NIOX MINO chemiluminescence analyser (Aerocrine, AB, Solna, Sweden) and according to the prevailing American Thoracic Society recommendations[22]. All measurements were taken prior to any forced manoeuvres or bronchial challenges as these tests may alter the FeNO measurement themselves. A sustained exhaled breath lasting at least 8 seconds was required with a flow rate of 50 ml/s as guided by automatic feedback from the device. Recordings were taken in triplicate with the mean value used for the reported result.

Due to the portability of the NIOX MINO device, it was additionally used as a domiciliary primary outcome measure for the first study in this thesis. Patients were instructed on its use, and they then took their own triplicate measures both morning and evening for the duration of the study.

SKIN PRICK TESTING

Skin prick testing (Merck, Middlesex, UK) was performed using 8 common aeroallergens in the relevant studies: trees, grass, house dust mite, weeds, feathers, aspergillus, cat and dog. Each allergen was injected subcutaneously to the volar aspect of a forearm; having withheld antihistamines for at least 72 hours. Saline (0.9% wt/vol) and histamine (1.7mg/ml) were also injected subcutaneously as negative and positive controls respectively. A positive response was defined by any

wheal whose diameter was 3mm greater than the negative control, 15 minutes after the application of allergen.

BLOOD AND URINARY BIOMARKERS

Peripheral blood measurements

Whole blood was taken by venepuncture from subjects prior to bronchial challenge procedures. Counts inclusive of blood eosinophil levels were measured using a Sysmex XE 2100 haematology autoanalyser (Sysmex UK Ltd, Bucks, UK). Separate samples were taken for biochemical analysis and measurement of Urea and electrolytes, liver function tests and random glucose. All blood samples were processed and analysed in the biochemistry department of Ninewells Hospital and Medical School, Dundee.

Serum eosinophilic cationic protein

After venepuncture, whole blood was rested for 60 minutes at 22 to 24^{o}C. It was then centrifuged for 10 minutes at 3000 rpm, at 4^{o}C. The supernatant was stored in a freezer kept at – 20^{o}C and analysed in batches upon study completion.

Serum ECP was measured in duplicate using a UniCAP immunoassay system (Phadia Ltd, Milton Keynes, UK) with a coefficient of variation (CV) of 3%. ECP measurements were performed in the department laboratory.

Overnight urinary cortisol/creatinine ratio

Overnight urine was collected between 22.00h and 08.00h. Urinary free cortisol was measured using a commercial RIA kit (DiaSorin, Dartford, Kent, UK). The intra-assay CV was 21%, with the inter-assay CV of 13%. Urinary creatinine was measured using the RATE technique on a Cobas-Bio auto-analyser (Roche Products, Sussex, UK), with an inter-assay CV of 4.3%. Urinary measurements were performed in the department laboratory.

QUALITY CONTROL

Sensitivity, specificity and coefficients of variation were monitored for individual batches of assays within the departmental laboratory. Measurements carried out within the main Ninewells Hospital haematology and biochemistry laboratories were subject to NHS quality standards.

STATISTICAL METHODS

Data were analysed throughout the studies using several iterations of SPSS statistical software for both Windows and Mac, the most recent of these is version 22 (SPSS Inc., USA). Power calculations have been derived within the relevant studies. For all studies, statistical significance for any comparison was deemed to have been achieved below an alpha-error of 5% (two-tailed), with 95% confidence intervals for the mean change given when appropriate. Graphical representation of data was produced using GraphPad Prism version 5 (Graphpad Software Inc., USA). Further statistical detail is provided within each study.

CHAPTER 3:

INHALED CORTICOSTEROID DOSE-RESPONSE USING DOMICILIARY EXHALED NITRIC OXIDE IN PERSISTENT ASTHMA - THE F_ENOTYPE TRIAL

Study Aims:

1. To evaluate the dose response to ICS in a phenotype-enriched sample of persistent asthmatics upon domiciliary FeNO measurements.
2. To assess how any FeNO response is related to other inflammatory outcome measures in asthma.
3. To assess how any FeNO response to an ICS dose ramp correlates with asthma control.

ABSTRACT

Background

International guidelines advocate a standard approach to asthma management for all despite its heterogeneity. 'Personalised' treatment for inflammatory asthma phenotypes confers superior benefits.

Objective

To evaluate dose-response to inhaled corticosteroids (ICS) in asthmatics with an elevated exhaled nitric oxide (FeNO) phenotype using domiciliary measurements.

Methods

We performed a randomised, crossover trial in 21 mild-to-moderate persistent asthmatics receiving ICS with elevated FeNO (>30ppb) that increased further (>10ppb) after ICS washout. Patients were randomised to 2 weeks of either fluticasone propionate 50µg twice daily (FP100) or 250µg twice daily (FP500). The primary outcome was response in diurnal domiciliary FeNO levels. Secondary outcomes included: mannitol challenge; serum eosinophilic cationic protein (ECP); blood eosinophil count; and asthma control questionnaire (ACQ).

Results

We found significant dose-related reductions of diurnal FeNO compared to baseline - morning FeNO: baseline=71ppb (95%CI: 61-83ppb); FP100=34ppb (95%CI: 29-40ppb), $p<0.001$; FP500=27ppb (95%CI: 22-33ppb), $p<0.001$; and significant dose separation for morning, $p<0.05$, and evening, $p<0.001$. Time series FeNO displayed

exponential decay: FP100 R^2=0.913, half-life=69hrs (95%CI:50-114hrs); FP500 R^2=0.966, half-life=55hrs (95%CI:45-69hrs); as well as diurnal variation. ACQ showed significant improvements exceeding the minimal important difference (>0.5) with values in keeping with controlled asthma (<0.75) after each dose: FP100=0.48 (95%CI:0.24-0.71), p=0.004; FP500=0.37 (95%CI:0.18-0.57), p=0.001. All other secondary inflammatory related outcomes (mannitol, ECP and eosinophils) showed significant improvements from baseline but no dose separation.

Conclusions

There is a significant dose-response of diurnal FeNO to ICS in asthmatics with an elevated FeNO phenotype, which translates into well-controlled asthma. Further interventional studies are warranted using domiciliary FeNO in this specific phenotype.

Ethics Reference: 09/S0501/52

MHRA reference: 21726/0268/001-0001

ClinicalTrials.gov reference: NCT00995657

INTRODUCTION

Asthma is a common pulmonary disease that is increasing in prevalence worldwide[177]. It is characterised by intermittent, reversible airflow obstruction due to bronchoconstriction following a variety of stimuli, which can progress to exacerbations and hospitalisations, with associated mortality. Inhaled corticosteroids (ICS) are widely recognised as the gold-standard asthma control treatment. However, asthma is notable for its heterogeneity of presentation; and responses to inhaled bronchodilator and inhaled corticosteroid therapy. Pointedly, there is no gold-standard diagnostic test for asthma; rather it is necessary to combine investigations with symptoms and signs to improve diagnostic accuracy[10].

Asthma heterogeneity is not reflected well in current national[10] and international[11] asthma guidelines which are based on symptoms and spirometry. Variability of asthma outcomes also exists, that can depend on the type and/or dose of inhaled corticosteroid[210], or parameters such as body mass index[3,180] and smoking status[175]. A variety of asthma phenotypes[211] are recognised including eosinophilic, neutrophilic, and non-eosinophilic steroid-unresponsive asthma[150]. Several landmark studies have examined 'personalising' asthma treatment based on clinical hallmarks of asthma - airway hyper-responsiveness (AHR)[13] and airway inflammation[6,14] – revealing that phenotype-driven asthma treatment confers benefits over standard care.

Fractional exhaled tidal nitric oxide (FeNO) can now be measured both simply and reliably. FeNO has previously been studied, with recent publication of guidelines[212] from the American Thoracic Society (ATS). Smith and colleagues[15] evaluated titrating ICS against a specific FeNO threshold (<15ppb), demonstrating that

maintenance ICS doses could be reduced without loss of asthma control. A separate study[213] found ICS doses increased when using FeNO in addition to standard care, with no difference in asthma control. However, FeNO levels vary significantly between patients[214].

We wished to evaluate the dose-response to ICS in a phenotype-enriched sample of persistent asthmatics *all* displaying elevated levels of FeNO when stepped off ICS; along with how these responses related to other inflammatory outcomes and, most importantly, asthma control. Pointedly, no studies to date have used domiciliary, diurnal measurements of FeNO for this purpose. We hypothesised that by using these stringent phenotypic criteria; any dose-response to ICS would be more predictable.

METHODS

Patients

Male and female patients aged 18-65 years, with mild-moderate persistent asthma, who were receiving 200-1000µg/day ICS (beclometasone dipropionate (BDP) equivalent), were screened (Figure 2). Recruited patients were required to have FeNO>30ppb with a further rise (>10ppb) following a two week washout from ICS. Exclusion criteria were: recent respiratory tract infection or oral corticosteroid use (<3 months); smoking within the previous year or >10pack-years smoking history.

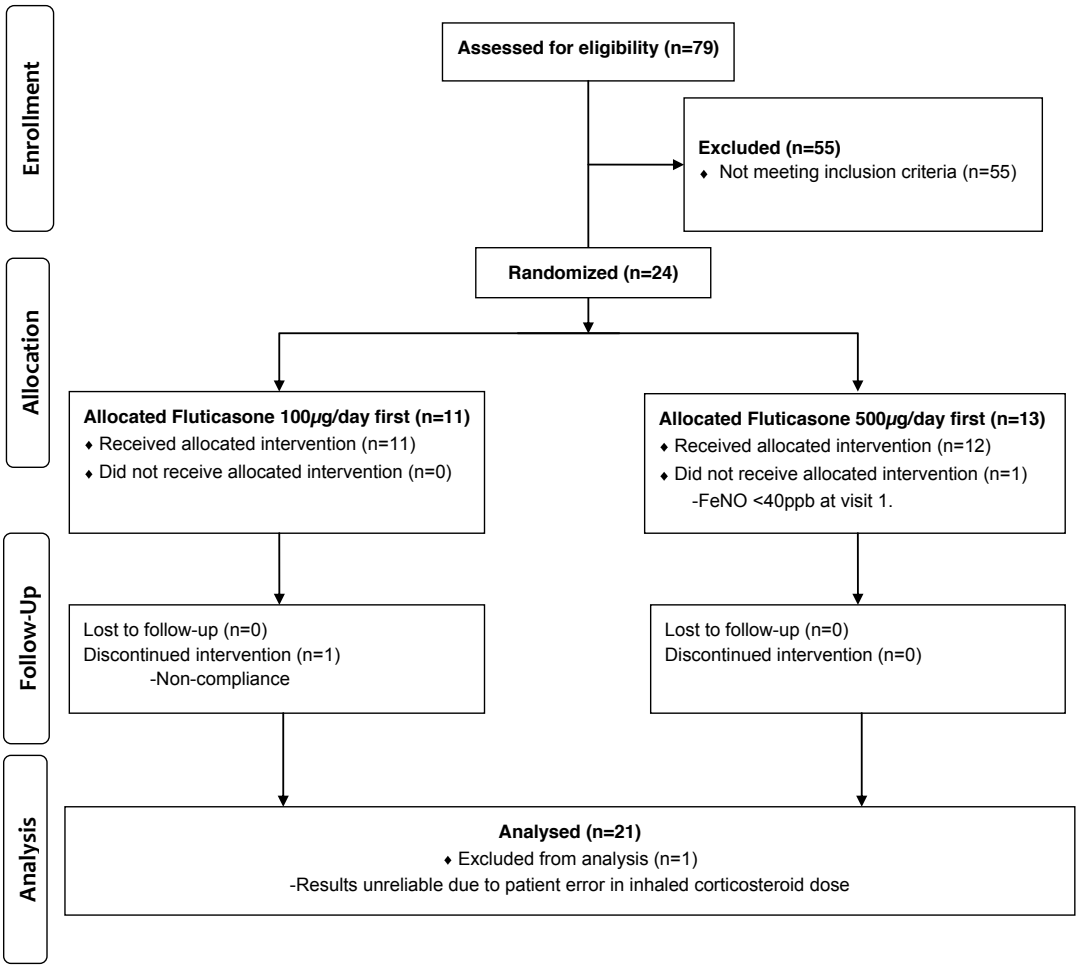

Figure 2. Consolidated Standards of Reporting Trials diagram.

FeNO, fractional exhaled nitric oxide; ppb, parts per billion.

Study Design

We performed a randomised, double blind, crossover study (ClinicalTrials.gov number: NCT00995657) of inhaled fluticasone propionate 50ug 1puff twice daily (FP100) and 250ug 1puff twice daily (FP500) each for two weeks via pressurised metered dose inhaler (Flixotide Evohaler, Allen and Hanburys, Uxbridge, UK), (Figure 3). After screening, patients were stepped off second-line medications: long-acting beta-agonists (LABA); theophyllines; leukotriene receptor antagonists; and converted to a fluticasone propionate equivalent of their usual ICS dose - stepping off this over two weeks followed by a two week ICS-free washout period. There was also a two-week ICS washout period between treatments. The primary outcome measure was change in daily morning and evening FeNO from post-washout baseline. Secondary outcomes were: domiciliary spirometry; laboratory-based spirometry; impulse oscillometry (IOS); AHR to mannitol challenge; asthma control questionnaire (ACQ) and mini asthma quality of life questionnaire (mini-AQLQ) scores; blood eosinophil counts and serum eosinophilic cationic protein (ECP).

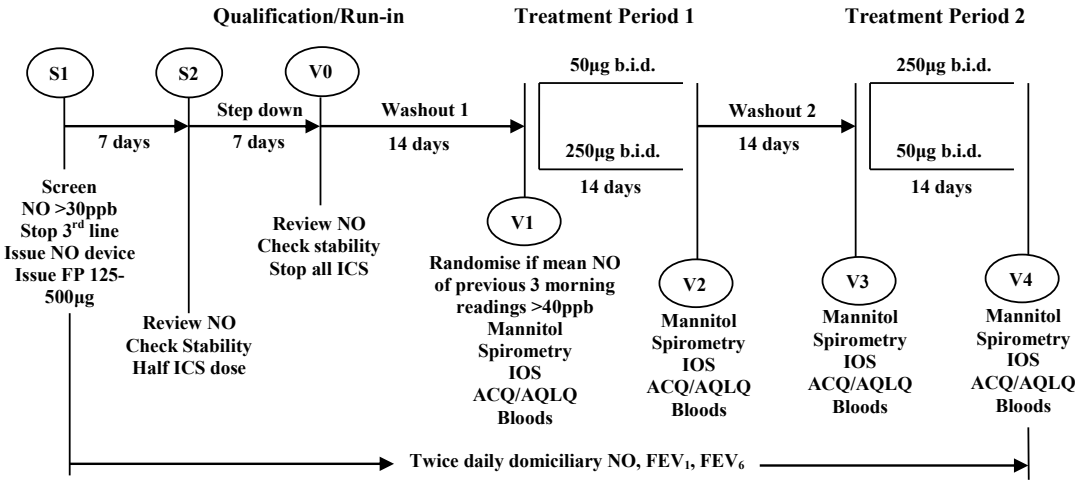

Figure 3. Study flow diagram.

ACQ, Asthma Control Questionnaire; AQLQ, mini-Asthma Quality of Life Questionnaire; FEV_6, forced expiratory volume in 6s; FP, fluticasone propionate; ICS, inhaled corticosteroid; Mannitol, mannitol bronchial challenge; S1, S2, screening visits; V0, pre-washout visit; washout periods are sequential (i.e., irrespective of FP dose); V1-4, study visits. See Figure 2 legend for expansion of other abbreviations.

Measurements

Domiciliary FeNO was performed morning and evening in the patient's home, in duplicate, using a handheld NIOX MINO (Aerocrine, Solna, Sweden) according to manufacturer's instructions and as previously described[215] at a standard flow rate (50ml/s). Domiciliary forced expiratory volume in 1s and 6s (FEV_1, FEV_6) were performed in triplicate morning and evening using a handheld PiKo-6 (n-Spire Health, Longmont, CO, USA) to manufacturer's instructions and after FeNO to avoid exaggerated FeNO measurements. Impulse oscillometry (Masterscreen IOS, Höchberg, Germany) was performed in triplicate to manufacturer's guidelines. A SuperSpiro spirometer (Micro Medical Ltd., Kent, UK) was used for spirometry in triplicate in accordance with European Respiratory Society guidelines[216]. Mannitol challenge was performed as previously described[217] with a dry powder inhaler (Aridol Pharmaxis Ltd, Sydney, Australia) using cumulative dose increments up to 635mg.

The cumulative provocative dose producing a 15% fall in FEV_1 (PD_{15}) was then calculated. The ACQ[33] and mini-AQLQ[35] were completed at study visits. Serum ECP was analysed in duplicate using a UniCAP system (Phadia, Milton Keynes, UK), coefficient of variation=3%. Peripheral blood eosinophils were measured using the Sysmex XE2100 Haematology autoanalyser.

Statistical Analysis

A power calculation determined 20 patients completing in a crossover fashion would ensure 90% power (two-tailed, alpha-error=0.05), to detect a 5.4ppb difference in FeNO between treatments (within-patient SD=5ppb).

All data were examined for normality, with non-Gaussian distributions logarithmically transformed. All baselines were pooled as no significant differences were demonstrated comparing values between the ends of each washout. Baseline and post-treatment values for domiciliary measures were the mean of days 12-14 from each period. Differences between group means were analysed using repeated measures analysis of variance with Bonferroni correction. Paired Student's t-tests were used for differences within or between groups as change from baseline.

Time series plots were created for domiciliary measures (FeNO, FEV_1, FEV_6). Non-linear regression was used to describe FeNO response in both treatments, with best-fit curves compared using the extra sum-of-squares F-test. Estimates of time to 25%, 50%, 75% and 90% reduction in FeNO from baseline were calculated from the exponential curve half-lives. Linear regression was used for time series changes in

domiciliary FEV_1 and FEV_6 for each two-week period. Harmonic analysis using a Cosinor model over 24hrs (similarly described for peak expiratory flow rate[218]) was applied to the plateau (week two) phases of each of pooled baseline and treatment group time series to assess for diurnal variation of the domiciliary measures. Diurnal variation amplitudes were derived from geometric mean fold differences in diurnal FeNO.

RESULTS

21 patients completed per protocol. Screening demographics were: mean age 36.7yr (95%CI: 31.0-42.5yr); 15 male; mean BDP equivalent dose 441µg/day (95%CI: 332-549µg/day); mean spirometry (FEV_1, FVC, FEF_{25-75}) as percentage of predicted were 94.5% (95%CI: 89.5-99.4%), 108.7% (95%CI: 104.7-112.6%) and 61.3% (95%CI: 54.1-68.6%) respectively; 17 patients had ≥1 positive skin prick test; 7, 1 and 8 patients were receiving LABAs, LTRAs and antihistamines respectively.

For the primary outcome of domiciliary FeNO, significant reductions after two weeks treatment were demonstrated within both FP100 and FP500 groups from pooled baseline, for both morning and evening values (Table 3); with significantly greater reduction in FeNO after the higher dose. Time-series plots of all mean morning and evening FeNO values were created for both FP100 and FP500 treatments (Figure 4) and both washout periods (Figure 5). Non-linear regression demonstrated that an exponential one-phase decay curve was the best-fit model for each of FP100 and FP500 time-series FeNO plots (Figure 4, Table 4). For FP100 the fit-of-curve coefficient of determination R^2=0.913, with a half-life ($t_{1/2}$)=69.8hrs (95%CI: 50.3-114hrs). For FP500: R^2=0.966; $t_{1/2}$=54.8hrs (95%CI: 45.4-68.9hrs). The exponential curves were significantly different ($p<0.001$), confirming the dose-response effect. There was significant diurnal variation of FeNO during each of the second (plateau) weeks (days 8-14) as follows: pooled washout (amplitude=15.6%; R^2=0.521, p=0.004); FP100 (amplitude=24.3%; R^2=0.412, p=0.01); and FP500 (amplitude=28.9%; R^2=0.439, p=0.01). Mean domiciliary FeNO measurements were almost exclusively higher in the morning.

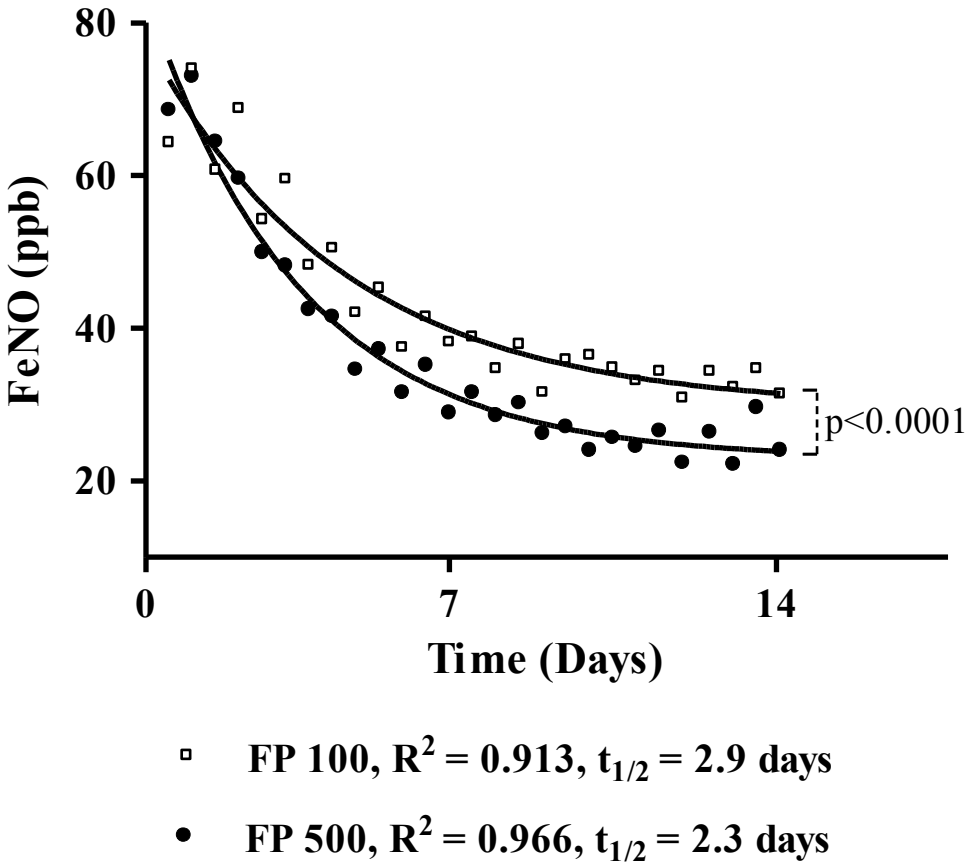

Figure 4. Time series morning and evening FeNO values and one-phase exponential decay curves.

FeNO values displayed as geometric means at each sequential time point for each group. R^2=coefficient of determination (goodness of fit) of exponential decay curves to each data set. $t_{1/2}$=half-life of exponential decay. ppb=parts per billion.

Table 3. Domiciliary and visit outcome measures.

	Pooled Baseline		FP 100µg daily		FP 500µg daily		FP 100 vs. FP 500	
	AM	PM	AM	PM	AM	PM	AM	PM
DOMICILIARY								
FeNO (ppb)*	71 (61, 83)	67 (56, 81)	34 (29, 40) †††	31 (27, 37) †††	27 (22, 33) †††	22 (19, 26) †††	0.04	<0.001
FEV_1 (Litres)	3.00 (2.65, 3.35)	3.15 (2.76, 3.54)	3.19 (2.80, 3.58) ††	3.21 (2.76, 3.66)	3.20 (2.79, 3.60) †	3.19 (2.77, 3.61)	0.99	0.99
FEV_6 (Litres)	3.90 (3.43, 4.37)	3.99 (3.50, 4.48)	4.15 (3.63, 4.67) †	4.05 (3.52, 4.59)	4.10 (3.64, 4.56)	4.07 (3.58, 4.56)	0.99	0.99
VISITS								
Inflammatory								
Mannitol PD_{15} (mg) *	102 (57, 180)		317 (178, 566) †††		418 (256, >635) †††		0.46	
Serum ECP (µg/L) *	18.6 (12.5, 27.8)		18.1 (11.6, 28.2)		13.0 (8.7, 19.6) ††		0.07	
Blood Eosinophils ($x10^9$/L)	0.37 (0.29, 0.45)		0.31 (0.23, 0.38)		0.28 (0.21, 0.34)		0.99	
Pulmonary Function								
IOS:								
R5 (% pred) *	118 (102, 138)		109 (95, 127)		109 (95, 126)		0.99	
F_{res} (kPa/L/s) *	13.9 (11.3, 17.2)		12.0 (10.1, 14.2)		11.9 (10.2, 13.8)		0.99	
Spirometry:								
FEV_1 (% pred)	89 (84, 94)		96 (91, 100) ††		96 (93, 99) †††		0.99	
FEF_{25-75} (% pred)	54 (46, 61)		62 (55, 70) †††		63 (56, 70) †††		0.99	
Questionnaires								
ACQ mean score	1.16 (0.79, 1.54)		0.48 (0.24, 0.71) ††		0.37 (0.18, 0.57) †††		0.30	
Mini-AQLQ scores:								
symptoms	5.77 (5.37, 6.17)		6.45 (6.20, 6.70) †††		6.60 (6.42, 6.78) †††		0.35	
activity	6.38 (6.07, 6.69)		6.64 (6.34, 6.95) ††		6.76 (6.60, 6.92) ††		0.68	
emotion	6.12 (5.75, 6.49)		6.46 (6.10, 6.82) †		6.56 (6.25, 6.86)		0.99	
environment	5.90 (5.47, 6.32)		5.97 (5.47, 6.46)		6.29 (5.87, 6.70) †		0.13	
overall score	6.03 (5.74, 6.31)		6.40 (6.18, 6.62) ††		6.57 (6.41, 6.73) †††		0.05	

Domiciliary data are means of days 12-14 from washout (baseline), and each treatment period for morning or evening. Visit data are fixed time point measurements. Data are presented as arithmetic means (95% confidence intervals) unless stated. *Geometric mean (95% confidence intervals). Significance levels are given for within groups (FP100 and FP500 columns): $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, $^{\dagger\dagger\dagger}p<0.001$; and between groups in the rightmost column (Repeated measures analysis of variance with Bonferroni correction). FP=fluticasone propionate. AM, PM=morning, evening. FeNO=exhaled tidal nitric oxide. ppb=parts per billion. FEV_1=forced expiratory volume in 1 second. FEV_6= forced expiratory volume in 6 seconds. PD_{15}=provocative dose of mannitol causing a 15% fall in FEV_1. ECP=eosinophilic cationic protein. IOS=impulse oscillometry. R5=resistance at 5Hz. %pred=percentage of predicted. F_{res}=frequency of resonance. FEF_{25-75}=forced expiratory volume between 25-75% of FVC. ACQ=asthma control questionnaire. Mini-AQLQ=mini asthma quality of life questionnaire.

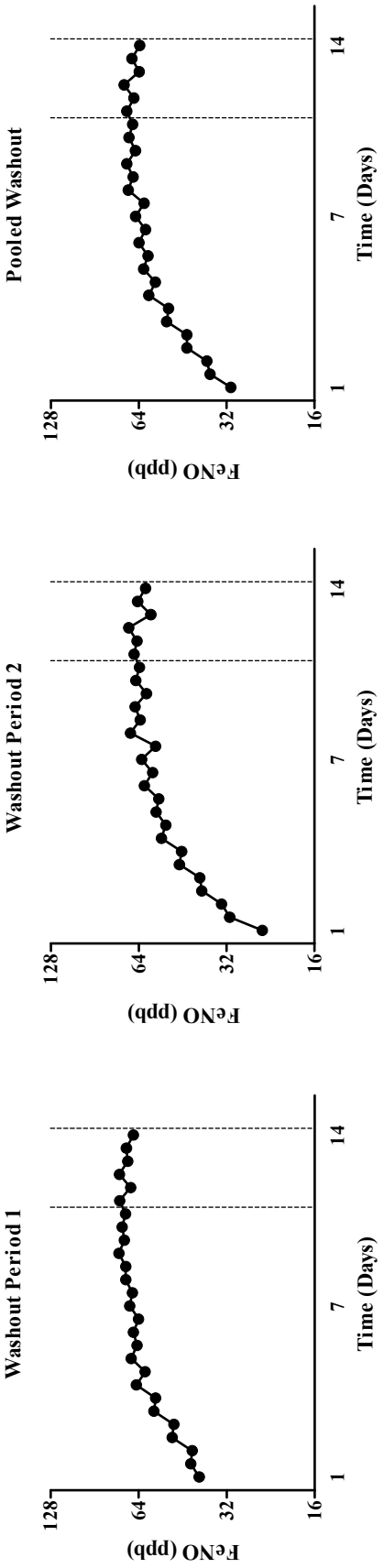

Figure 5. Sequential washouts 1&2 with pooled washout time-series for FeNO.

Values are geometric means of sequential diurnal FeNO levels. No significant difference between overall means of days 12-14 for each period (interrupted lines). ppb=parts per billion.

Table 4. Exponential one-phase decay time for domiciliary FeNO.

	Decay Time (Days)	
% Fall in FeNO	**FP 100µg daily** (R^2 = 0.91*)	**FP 500µg daily** (R^2 = 0.97*)
25	**1.2** (0.9, 2.0)	**0.9** (0.8, 1.2)
50 ($t_{1/2}$)	**2.9** (2.1, 4.8)	**2.3** (1.9, 2.9)
75	**5.8** (4.2, 9.5)	**4.6** (3.8, 5.7)
90	**9.7** (7.0, 15.8)	**7.6** (6.3, 9.5)

One phase exponential decay curves for FeNO significantly different between doses of fluticasone propionate (FP) each administered over 2 weeks ($p<0.0001$). R^2=coefficient of determination to exponential curve, *$p<0.001$. Data presented as time to percentage fall in FeNO from pooled baseline (estimated 95% confidence intervals). $t_{1/2}$=half-life of one phase exponential decay.

Domiciliary spirometry demonstrated significant improvements in morning FEV_1 as change from baseline within both FP100: 0.18L (95%CI: 0.1-0.26L), $p<0.001$; and FP500: 0.18L (95%CI: 0.08-0.29L), $p=0.004$; but no significant differences in evening FEV_1 at either dose, nor between groups for either morning or evening FEV_1 (Table 3). Time-series morning and evening FEV_1 (Figure 6) demonstrated significant linear patterns during pooled washout (FEV_1 reduction=0.21L, R^2=0.577, $p<0.001$) and both FP100 (FEV_1 increase=0.08L, R^2=0.157, $p=0.04$) and FP500 (FEV_1 increase=0.08L, R^2=0.262, $p=0.006$). Results for domiciliary FEV_6 are presented in Table 3. There was no significant diurnal variability of domiciliary spirometry.

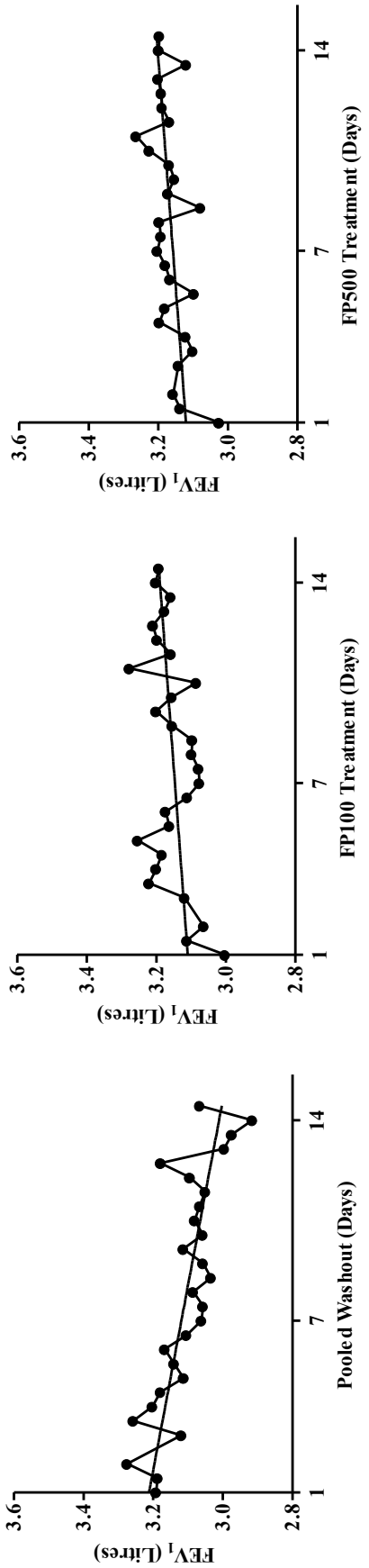

Figure 6. Time series for diurnal domiciliary FEV_1.

Sequential morning/evening mean FEV_1 over two weeks for pooled washout (left), fluticasone propionate (FP) 100ug/day (middle), and FP 500ug/day (right). Superimposed solid lines represent linear regression slopes.

Mannitol PD_{15} demonstrated significant improvements with both FP100 and FP500 as doubling-dose shifts from baseline, with no difference between doses (Table 3, Figure 7a). There was significant reduction in serum ECP after FP500 but not FP100 (Table 3, Figure 7b). Blood eosinophils were significantly reduced by both doses as change from baseline, with no difference between doses (Figure 7c). For IOS outcomes, there were no significant changes for any variable at either dose. For laboratory visit-based spirometry outcomes, both FEV_1 and FEF_{25-75} demonstrated significant improvements from baseline, with no differences between doses (Table 3, Figure 8). FVC did not change significantly at either dose.

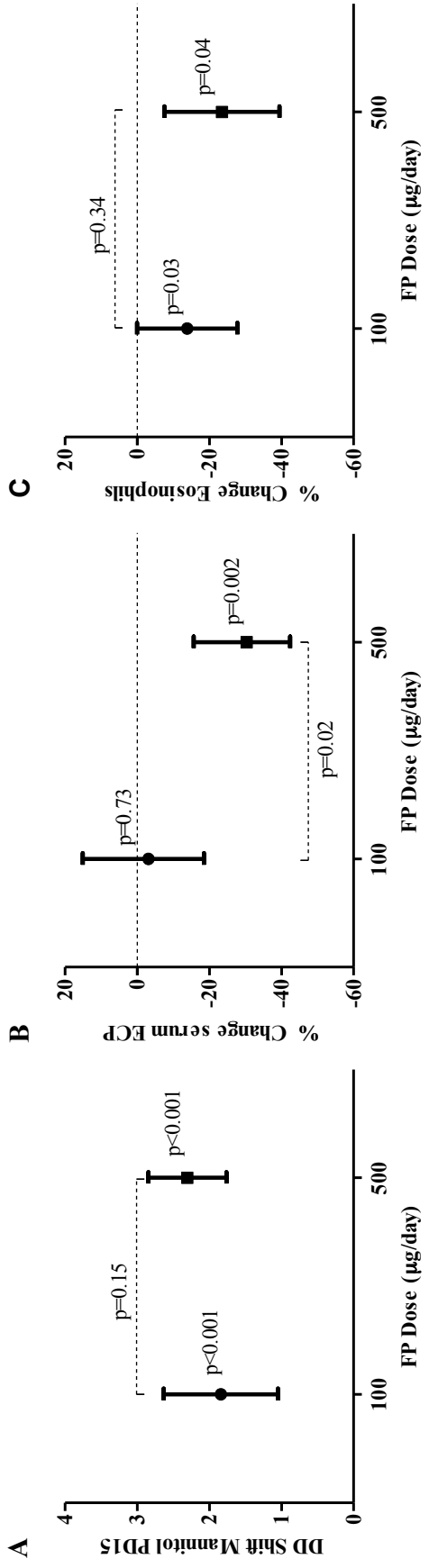

Figure 7. Secondary inflammatory outcomes.

(a) Mean doubling dose (DD) shift from baseline within groups for mannitol provocative dose causing 15% fall in forced expiratory volume in one second (PD_{15}). (b) Percentage changes within groups from baseline for serum eosinophilic cationic protein (ECP). (c) Percentage changes within groups from baseline for peripheral blood eosinophil counts. Error bars represent 95% confidence intervals.

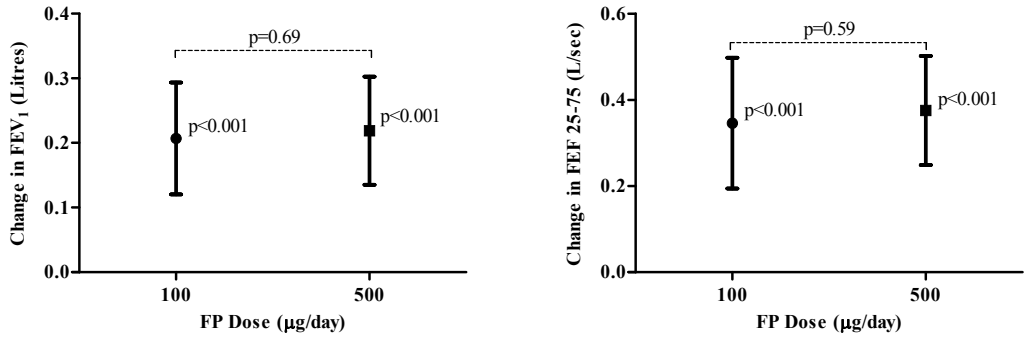

Figure 8. Standard visit spirometry.

Mean within group differences after 2 weeks treatment from pooled baselines for: Forced Expiratory Volume in 1 second (FEV1) (left) and Forced Expiratory Flow between 25-75% of FVC (FEF 25-75) (right). FP = fluticasone propionate. All error bars equate to 95% confidence intervals. P-values are for within group difference as absolute change from pooled baseline using Student's T-test for paired samples. Interrupted connecting lines represent between group differences compared by Student's T-test for paired samples. Significance set at $p<0.05$. ns = not significant.

ACQ score reduced significantly after 2 weeks in both groups from baseline with mean change exceeding the minimal important difference (>0.5), with absolute values after both FP100 and FP500 in keeping with well-controlled asthma (<0.75), (Table 3, Figure 9a). Mini-AQLQ overall score also improved significantly with both fluticasone doses from baseline (Table 3, Figure 9c). By separating the mini-AQLQ components, the same significant pattern was demonstrated for 'symptoms' (Table 3, Figure 9b).

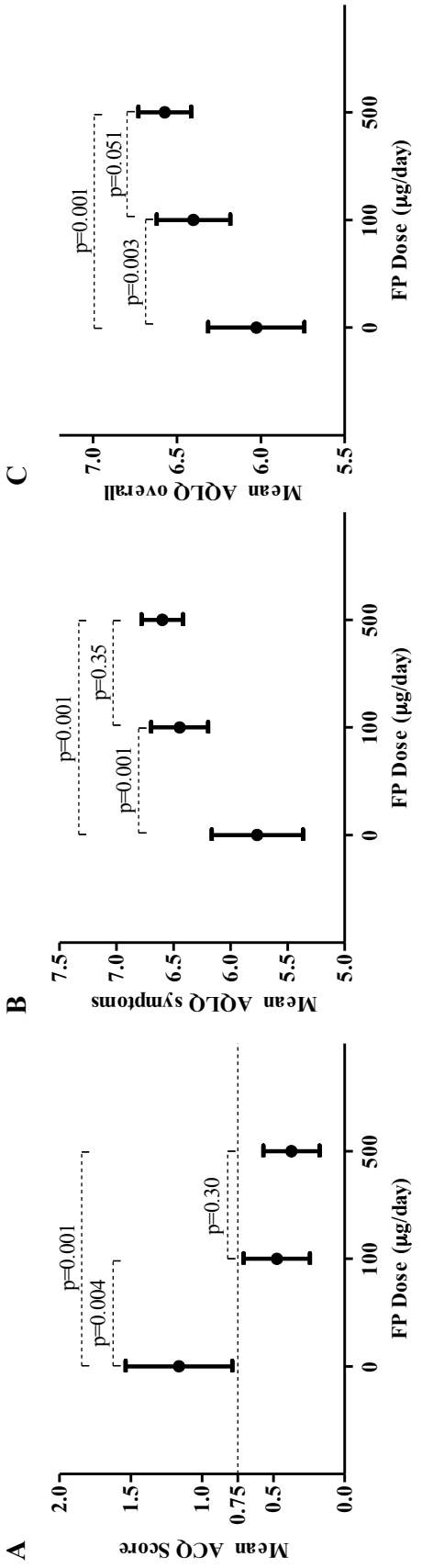

Figure 9. Asthma control and quality of life outcomes.

(a) Mean Asthma Control Questionnaire (ACQ) score at baseline, 100µg/day, and 500µg/day fluticasone propionate (FP). (b) Mean Asthma Quality of Life Questionnaire (AQLQ) symptom score at baseline, FP100µg/day, and FP500µg/day. (c) Mean Asthma Quality of Life Questionnaire (AQLQ) overall score at baseline, FP100µg/day, and FP500µg/day. Error bars represent 95% confidence intervals.

DISCUSSION

The present study has shown a significant dose-response relationship between diurnal FeNO and incremental ICS dosing (fluticasone propionate 100ug/day vs. 500ug/day) in asthmatics expressing an elevated FeNO inflammatory phenotype. FeNO responses to both doses demonstrate predictable one-phase exponential decay; with significantly faster decline, and greater suppression of FeNO at plateau, with the higher ICS dose. Furthermore, we have demonstrated significant diurnal variability of FeNO levels (higher in the morning) with or without ICS therapy.

This is the first study to have evaluated response of serial domiciliary diurnal FeNO in a dose-ranging manner with ICS. A previous study by Silkoff et al.[159] demonstrated a dose-response effect in asthmatics with high baseline FeNO levels using three separate doses of BDP, showing dose separation on FeNO between only the lowest (100μg) and highest (800μg) doses; albeit each treatment period was only for 1 week. However, that study did not examine the effect of FeNO reduction on asthma control; did not use domiciliary FeNO; and moreover used methacholine rather than mannitol challenge. Other studies have examined FeNO either in 'all-comers' with asthma[219], or asthmatics with near-normal baseline FeNO[160,219] thus reducing any signal from this as an outcome; despite asthmatics with high FeNO (>50ppb) being more likely to respond to ICS than those with low FeNO (<25ppb)[210,220]. In one study[221] with extra-fine hydrofluoroalkane (HFA) beclometasone, there was a plateau response at 100μg/day, although mean baseline levels were only 33ppb, compared to 71ppb in this study. Around 30% of the patients we screened for our study demonstrated high FeNO levels, even with ICS prescription; i.e. this phenotype may represent a moderately large proportion of asthma overall. Our FeNO response quantification

was augmented by portable analysers in the community, an approach used before in children to monitor home FeNO[215], but never before in adult asthmatics or for the prospective assessment of treatment response.

We already know that personalising asthma treatment according to phenotypes has proven benefits on symptoms, exacerbations and asthma control over-and-above standard care. The AMPUL study[13] demonstrated that by guiding asthma treatment, using AHR to methacholine, added to symptoms and lung function, greater asthma control could be achieved. Green et al[14] investigated using sputum eosinophil counts to guide anti-inflammatory therapy, demonstrating an overall reduction in exacerbations. Recently, we have utilised AHR to mannitol to guide ICS therapy[6], showing a reduction in mild exacerbations over 12 months, along with significant improvements in methacholine AHR, salivary ECP, FeNO, symptoms and reliever use.

Our domiciliary FeNO findings are supported by secondary (inflammatory) outcomes. Mannitol PD_{15} significantly improved with both doses of ICS in line with improvement in FeNO as previously described[222], however, mannitol could not discriminate between ICS doses; i.e. mannitol displays 'assay sensitivity' for ICS treatment response, with a 1.84 doubling-dose shift at the lower dose, but no evidence of dose-response sensitivity. However, stratifying patients to a mannitol phenotype (e.g. mannitol PD_{15} <100mg) rather than FeNO at study entry may alter this. For example, in a study powered on methacholine AHR there was a significant difference in response comparing 500ug vs. 100ug daily of HFA-fluticasone[223]. Blood eosinophils and ECP also reduced with ICS but only at higher dose for ECP,

suggesting this does not exhibit sufficient assay sensitivity. We chose our doses of fluticasone for this study based on previous dose-response data[224,225] and the systemic safety of no significant cortisol suppression at the higher dose (500μg/day)[226,227]. Interestingly in a meta-analysis of studies with fluticasone, a plateau response was seen at 200μg/day for various outcomes, although no inflammatory outcomes were evaluated[225].

We also demonstrated significant diurnal variability of domiciliary FeNO levels, both during ICS washout and following treatment with both doses of fluticasone, suggesting that FeNO may be additionally controlled by factors other than local airway inflammation. Pointedly, we are the first to show that diurnal variability of FeNO persists following incremental ICS dosing.

In terms of potential clinical relevance, the dose-response of FeNO to incremental ICS mirrored improvements in both asthma control and symptoms as measured by the ACQ and mini-AQLQ respectively. Indeed the baseline ACQ score demonstrated 'borderline' asthma control with the 95%CI lower bound >0.75, below which delineates good asthma control[34]. The ACQ score improved significantly with both doses of ICS to mean scores that were below the 0.75 level (including upper 95%CI), while the mean change exceeded the minimum important difference of 0.5[168]. Similarly, significant improvements were seen in the mini-AQLQ symptom component and overall score with both ICS doses. These are strong, clinically relevant signals for such a short study.

Significant but clinically unimportant changes were seen in all measurements of pulmonary function (domiciliary and laboratory). This is unsurprising given that

baseline lung function (spirometry; IOS) was relatively well preserved in these patients, and has reduced sensitivity to change over short time periods[125].

One limitation of this study was the short duration of ICS treatment, albeit this was a proof-of-concept study. However, there was still a significant and clinically important signal from the primary outcome of domiciliary FeNO; along with clinically significant improvements in symptoms and asthma control. Moreover, previous data shows that with AHR, a plateau response occurs after two weeks of treatment with fluticasone[225]. There will be bias in phenotype-driven analyses, but we designed this *a priori*, given the demands for 'personalised' treatment. One of the strengths of the present study was the novel use of diurnal domiciliary FeNO to increase the signal-to-noise ratio, which may also have had a serendipitous effect on bolstering compliance. Furthermore, we used additional inflammatory markers to validate this phenotype. While presently the cost of domiciliary FeNO is prohibitive, it is possible that with developing technologies this cost will come down.

In conclusion, we have demonstrated a significant dose-response relationship to ICS using diurnal domiciliary FeNO measurement in asthmatics with elevated FeNO as a common inflammatory phenotype. These responses follow predictable exponential decay that is significantly greater with higher ICS dosing. These findings translated into significant improvements in symptoms and asthma control in this selected phenotype despite the short study duration. Further studies are warranted in this specific asthma phenotype using longer-term outcome measures of asthma (e.g. exacerbations) following treatment based on monitoring domiciliary FeNO levels.

CHAPTER 4:

DOES BODY MASS INDEX INFLUENCE RESPONSIVENESS TO INHALED CORTICOSTEROIDS IN PERSISTENT ASTHMA?

Study Aims:

1. To examine for any putative relationship between obesity and the dose response to inhaled hydrofluoroalkane budesonide on a variety of asthma outcome measures.
2. To generate hypotheses for areas of further prospective evaluation with regard to inhaled corticosteroid dose response in overweight and obese individuals.

ABSTRACT

Background

While the relationship between asthma and obesity has been extensively explored, the effect of body mass index (BMI) on the dose response relationship to inhaled corticosteroids (ICS) has received little attention.

Objective

To assess the dose response of inhaled budesonide on outcome measures of asthma between overweight and normal weight persistent asthmatics.

Methods

72 mild-moderate persistent asthmatics from a post hoc analysis of previously reported trial data were divided into two groups: overweight, BMI≥25kg/m^2; normal weight, BMI<25kg/m^2. Each group received 4 weeks treatment with inhaled (hydrofluoroalkane) budesonide 200µg/day then 800µg/day with ICS washout pre-treatment. Outcome measures included FEV_1, Fractional exhaled nitric oxide (FeNO), Methacholine PC_{20}, total daily asthma symptom score, and overnight urinary cortisol/creatinine ratio were performed at baseline and after each dose.

Results

There were significantly greater improvements in the normal weight group for both FeNO and symptom responses at 0-200µg and 0-800µg ICS doses (as change from baseline), compared to the overweight group: FeNO 0-200µg, p=0.002; 0-800µg, p=0.045; Symptoms 0-200µg, p=0.002; 0-800µg, p=0.013. There was also a trend

towards attenuated cortisol suppression in overweight subjects at 0-800ug (p=0.06), but there was no significant difference at either dose in FEV_1 and methacholine PC_{20} between weight groups.

Conclusion

Overweight persistent asthmatics may have attenuated symptom and FeNO dose responses to inhaled budesonide compared to normal weight asthmatics, with no differences in FEV_1 or methacholine PC_{20} between groups. Attenuated cortisol suppression in the overweight group may be the clue to this difference, alluding to reduced peripheral lung deposition or absorption in overweight asthmatics.

Ethics reference: 08/S1402/14

EudraCT number: 2008-001027-59

ClinicalTrials.gov reference: NCT00667992

INTRODUCTION

It is widely accepted that the prevalence of both asthma[228] and obesity[229,230] are increasing. Obesity has also been proposed as a risk factor for a higher prevalence of asthma[178]. It follows, that increasing numbers of asthmatics will be overweight, necessitating a greater knowledge of any association, in particular in response to asthma medication. Many studies have explored the relationship between asthma and obesity. In general, these have suggested: that obesity makes one more likely to develop asthma[178]; obesity is related to worsening asthma control[231]; clinical measures of asthma (e.g. airway hyper-responsiveness [AHR], fractional exhaled nitric oxide [FeNO]) are affected by obesity[232-234]; and treatment of overweight asthmatics with standard regimens may be attenuated[235]. What is clear is that any relationship between asthma and obesity is complex and unlikely to be fully explained by one particular theory.

Despite the interest in this area, no direct attention has been given to the clinical effect of obesity on asthmatics' response to incremental doses of inhaled corticosteroids (ICS), the mainstay of asthma control treatment. Sutherland et al studied *in vitro* responses to glucocorticoids in overweight/obese asthmatics[236]. They found markers of dexamethasone response (including tumour necrosis factor [TNF]-α) were blunted - alluding to reduced clinical efficacy of glucocorticoid therapy. There have also been *post hoc* analyses of clinical trials data examining this. Peters-Golden et al studied the effect of body mass index (BMI) on percentage of asthma control days (ACD) between three groups of asthmatic patients receiving different asthma control medications at static doses (Montelukast 10mg/day, Beclomethasone 400µg/day, or Placebo)[237]. They found normal BMI asthmatics had a higher percentage of ACD

than overweight/obese asthmatics. The effect of beclomethasone on ACD was blunted with increasing BMI, whereas the montelukast effect remained constant across the BMI range. Curiously, the effect of placebo on ACD also decreased with increasing BMI making the authors question how much of the beclomethasone response could be related to placebo effect. There were no significant differences between BMI groups, by treatment for forced expiratory volume in 1 second (FEV_1) in this study. Boulet and Franssen studied response to inhaled fluticasone (200μg/day) as either a single agent or in combination with salmeterol and whether these were altered by BMI in 1242 currently steroid naïve asthmatics[235]. For both treatments they found the odds of achieving asthma control (including symptoms, rescue medication, morning peak flow, emergency visits and exacerbations) were significantly lower in obese patients, particularly with a BMI≥40kg/m^2. In summary, therefore, previous studies have examined the 'all or nothing' effect of ICS at a fixed dose on asthma outcomes in overweight/obese asthmatics, rather than true dose-response to ICS in a stepwise manner.

One also needs to consider the potential differential effects of drug pharmacokinetics and pharmacodynamics between obese and normal weight individuals. In general more lipophilic drugs have a larger volume of distribution in the obese, potentially reducing their peak effect but because they are then slowly released from the systemic tissues, can be present much longer. However, given that most ICS moieties deliver their effects at a local level in the lungs, any systemic absorption of ICS is likely to be low, unless the drug is very lipophilic or by using very high doses of ICS[49]. For budesonide (Table 1), its inhaled bioavailability is 15-30% with oral bioavailability around 11% (much higher than for example fluticasone at 1% oral bioavailability).

Budesonide is 88% protein bound, where again other ICSs are closer to 99%. It also has a short half-life of elimination and faster lung clearance than most ICS. It is, however, much less lipophilic than fluticasone. These properties may therefore mean that budesonide is more widely distributed systemically in the obese asthmatic, than their normal weight counterparts.

In this study we wished to examine the putative relationship between obesity and dose response to inhaled hydrofluoroalkane (HFA) budesonide through a variety of outcome measures – FEV_1 (airway calibre), FeNO (airway inflammation), methacholine bronchial challenge (AHR), and asthma symptom scores. We have taken advantage of previously reported clinical trials data that demonstrated equivalence between the dose response to both HFA and CFC budesonide in persistent asthmatics, to enable direct comparisons of dose response to ICS with BMI. We recognise that this method of study can only generate hypotheses for further investigation; nonetheless we believe it is an important and clinically relevant question.

METHODS

We performed a *post hoc* analysis of the HFA-budesonide data from a previously reported double-blind randomised controlled crossover trial, which compared HFA and chlorofluorocarbon (CFC) formulations of inhaled budesonide at doses of 200ug and 800ug/day (as compared to ICS-free baseline) in persistent asthmatics[2]. The trial demonstrated no difference in response to HFA-budesonide given first or second in randomised sequence order, and importantly, no difference in baseline values after each washout period. We elected not to include data from the CFC-budesonide arm, because this would have resulted in using values from the same patient twice for each outcome measure, thus potentially biasing the results. Moreover, as CFC-budesonide is no longer available, the HFA-budesonide data is more relevant to current prescribing practice.

The reference trial was a single-centre, randomised, crossover study of HFA and CFC budesonide pressurised metered dose inhaler (pMDI) formulations. The institutional review board approved the study and all participants gave informed consent. Eligible participants aged 18–65years had a diagnosis of stable, persistent asthma receiving ≤1000μg beclomethasone dipropionate or equivalent. Eligible patients entered a step-down phase, during which second-line controllers were withdrawn, i.e. long-acting $beta_2$-agonists, theophyllines, cromones and leukotriene inhibitors. Patients then had their usual ICS dose tapered in a manner similar to previously published studies from this department[238]. They then entered a 1-week pre-treatment (ICS-free) washout period. A methacholine provocative concentration causing a 20% decrease in FEV_1 (PC_{20}) of ≤4mg/ml and FEV_1≥60% predicted after step-down was required for entry

to randomisation. Subjects not meeting the entry requirements at the end of this phase were given a further 1–2 weeks of washout.

The reference trial consisted of two randomised treatment periods. Patients first received a low-dose (200μg/day) of budesonide, either via CFC or HFA pMDI, for 2 weeks, followed by 2 weeks of medium-dose (800μg/day) budesonide of the same formulation. Participants then crossed over treatment arms to the remaining budesonide formulation using the same dosing regimen; effectively acting as their own control. Treatment periods were separated by a 1–2 week ICS-free washout period. Subjects were required to be within one doubling dilution of baseline methacholine PC_{20} at the end of washout.

During all study visits, participants underwent pulmonary function testing, exhaled tidal nitric oxide measurement and methacholine challenge. Methacholine challenge was performed using the five-breath dosimeter technique in accordance with American Thoracic Society (ATS) recommendations[20]. The PC_{20} was calculated using log-linear interpolation of the dose–response curve[20]. Exhaled tidal nitric oxide was measured in line with ATS recommendations using a NIOX analyser (NIOX® Nitric Oxide Monitoring System, Aerocrine AB, Solna, Sweden). Subjects were asked to rate their asthma symptoms twice daily; daytime scores recorded each evening, and night-time scores on waking. The following rating scales were used: 0=no asthma symptoms; 1=mild symptoms (easily tolerated); 2=moderate symptoms (interferes with normal activities/sleep); and 3=severe (prevents normal activities/ sleep). Overnight (22.00–08.00h) urine was collected for cortisol and creatinine at baseline and the night prior to study visits. Urinary cortisol was measured using a

commercial radioimmunoassay kit (DiaSorin Ltd, Wokingham, Berks, UK). The intra-assay coefficient of variation=4%, with inter-assay coefficient of variation=8%. Urinary creatinine was measured on a Cobas-Bio auto analyser (Roche Products, Welwyn Garden City, Herts., UK). The intra-assay and inter-assay coefficients of variation were 4.6% and 3.0%, respectively. Skin prick testing to a battery of common aeroallergens (Merck, UK) was performed at baseline.

Statistical Analysis

Data were assessed for normality using Kolmogorov-Smirnov tests along with visual inspection of data on histograms and Q-Q plots. Non-Gaussian data were logarithmically transformed to achieve normality prior to analysis or analysed using non-parametric tests if normality could not be achieved with transformation. Results data from log transformed values after analysis were then anti-logged and presented either as geometric means (for absolute values), geometric mean fold differences or percentage changes (for within and between group differences). A multivariate analysis of variance (MANOVA) was performed to assess for any cumulative Type-I errors inherent with multiple T-tests as well as interactions between the different outcome variables with significance set at $p<0.05$ (two-tailed). Pearson's and Spearman's correlations were performed on significant outcome variables from the between group T-tests or Mann-Whitney-U tests respectively, with BMI as a continuous (rather than categorical) variable. Differences of *within* group means compared to respective baselines were assessed using paired Student's T-tests (2-tailed) or related-samples Wilcoxon Signed Ranks tests. Differences *between* group means were assessed using independent Student's T-tests (2-tailed) or independent-

samples Mann-Whitney-U tests. All significance levels were set at $p<0.05$. Statistical analyses were performed using SPSS version 18.0.

RESULTS

A total of 72 patients (95% white Scottish) were identified combining the two HFA-budesonide arms. Two independent groups were subsequently identified based on their BMI; an overweight group with BMI≥25kg/m^2 (mean 30.0kg/m^2, range 25.1–40.5kg/m^2) and a normal weight (normal) group with BMI<25kg/m^2 (mean 22.9kg/m^2, range 18.9–24.9kg/m^2). The baseline demographics for these two groups are presented in Table 5. Of note, the overweight group were significantly older by approximately 9 years, with the normal group having a significantly higher baseline FeNO by approximately 13 parts per billion. Subsequent Pearson's correlation, however, demonstrated no significant effect of age (p=0.30) or baseline FeNO (p=0.24) with BMI as a continuous variable. There were no significant differences between remaining baseline values.

Table 5. Baseline demographics.

Group	Overweight BMI≥25 (n=45)	Normal Weight BMI<25 (n=27)	p-value
Sex (M:F)	21:24	8:19	0.15
Age (years)	42.8 (39.0-46.6)	34.3 (28.6-39.9)	0.01
Weight (kg)	86.4 (82.9-90.0)	64.15 (60.9-67.4)	<0.001
Height (cm)	169.8 (167.3-172.2)	167.0 (163.6-170.5)	0.20
Body Mass Index, BMI (kg/m^2)	30.0 (28.9-31.1)	22.9 (22.4-23.4)	<0.001
Atopic status, ≥ 1 positive skin prick (%)	93.3	96.3	0.60
FEV_1 (L)	2.83 (2.66-3.01)	2.83 (2.54 -3.13)	0.99
FEV_1 (% predicted)	82.1% (78.4-85.8)	81.7% (78-85.3)	0.87
FeNO (ppb)*	29.7 (24.1-36.6)	43.2 (33.5-55.7)	0.03
Methacholine PC_{20} (mg/ml)*	0.80 (0.6-1.07)	0.61 (0.43-0.87)	0.27
Total daily symptom score (0-3)	0.94 (0.73-1.16)	0.98 (0.73-1.23)	0.81
Cortisol/Creatinine Ratio (nmol/mmol)*	3.38 (2.72-4.21)	3.72 (2.8-4.95)	0.60

Data presented as arithmetic mean (95% confidence intervals) unless stated. *Geometric mean (95% confidence interval). FEV_1= Forced expiratory volume in 1 second. FeNO= Fractional exhaled nitric oxide. PC_{20}= Provocation concentration (methacholine) causing 20% fall in FEV_1 from baseline. Significance level p<0.05. ppb= parts per billion.

Significant differences were observed between overweight and normal groups for both FeNO and total daily asthma symptom scores. For FeNO (Table 6), there was a significantly greater reduction for 0-200µg and 0-800µg budesonide in the normal group than in the overweight group (Figure 10, Plot D), albeit the normal group started from a higher baseline. Most of the reduction in FeNO was between 0-200µg of budesonide. A similar pattern was seen with symptoms (Table 7), with significantly greater improvement in symptoms in the normal group for 0-200µg and 0-800µg of budesonide (Figure 10, Plot B). Again most symptom improvement was between 0-200µg.

Table 6. Fractional exhaled nitric oxide (FeNO).

	Overweight		**Normal Weight**		**Difference**	
	Mean (95%CI)	P	Mean (95%CI)	P	Mean (95%CI)	P
Baseline	29.7 (24.1, 36.6)*		43.2 (33.5, 55.7)*		0.69 (0.49, 0.96)	0.03
0-200µg	0.74 (0.67, 0.82)	<0.001	0.56 (0.48, 0.65)	<0.001	1.32 (1.11, 1.57)	0.002
0-800µg	0.63 (0.54, 0.74)	<0.001	0.48 (0.40, 0.58)	<0.001	1.30 (1.01, 1.67)	0.045
200-800µg	0.85 (0.76, 0.95)	0.005	0.87 (0.74, 1.01)	0.08	0.98 (0.81, 1.18)	0.83

Data presented as geometric means (parts per billion)* for baseline values and geometric mean fold differences (95% confidence intervals) from baseline vs. 200µg (0-200µg), baseline vs. 800µg (0-800µg), 200µg vs. 800µg (200-800µg), and between groups of ICS. For each variable p-values (P) are shown for both within group responses and for between group differences in response. Significance level $p<0.05$. ICS= Inhaled corticosteroid (HFA-budesonide).

Table 7. Total daily asthma symptom scores.

	Overweight		Normal Weight		Difference	
	Mean (95%CI)	P	Mean (95%CI)	P	Mean (95%CI)	P
Baseline	0.94 (0.73, 1.16)*		0.98 (0.73, 1.23)*		-0.04 (-0.39, 0.31)	0.78
0-200µg	-0.10 (-0.29, 0.09)	0.11	-0.53 (-0.70, -0.35)	<0.001	0.43 (0.14, 0.71)	.002
0-800µg	-0.29 (-0.53, -0.06)	0.002	-0.69 (-0.90, -0.48)	<0.001	0.40 (0.04, 0.75)	0.01
200-800µg	-0.20 (-0.41, 0.01)	0.007	-0.16 (-0.31, -0.02)	0.009	-0.03 (-0.33, 0.27)	0.98

Data are presented as arithmetic means* for baseline values and arithmetic mean changes (95% confidence intervals) from baseline vs. 200µg (0-200µg), baseline vs. 800µg (0-800µg), 200µg vs. 800µg (200-800µg), and between groups of ICS doses. For each variable p-values (P) are shown for both within group responses and also for the between group differences in response. Significance level $p<0.05$. ICS= Inhaled corticosteroid (HFA-budesonide).

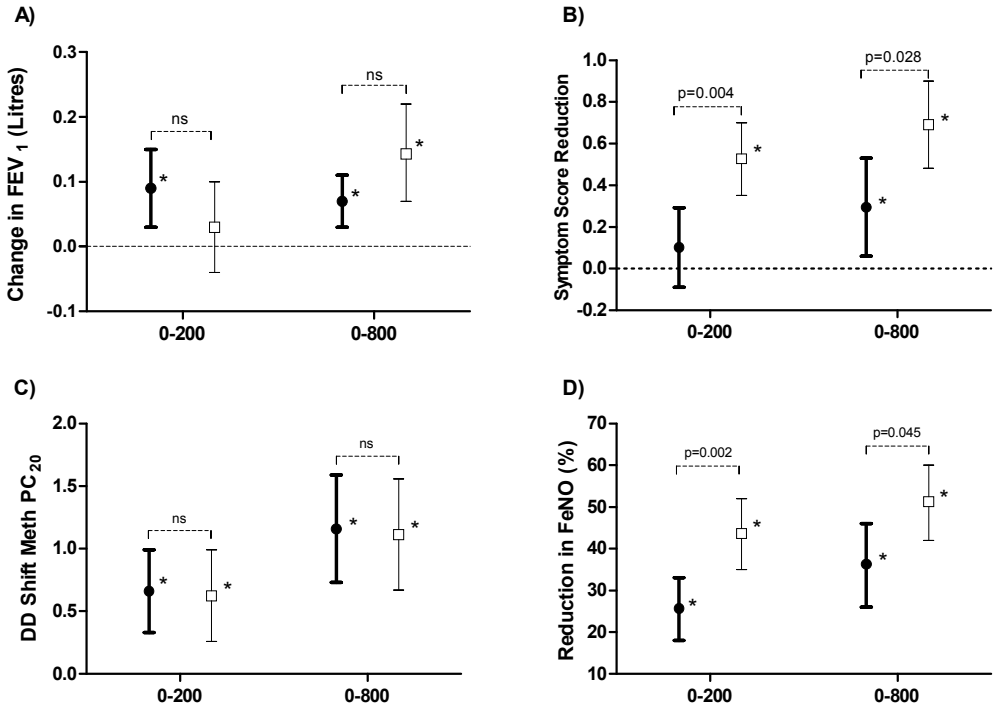

Figure 10. Between group differences by body mass index for 0-200µg and 0-800µg daily inhaled HFA budesonide dose increments.

A) Mean change in FEV_1 from baseline. B) Mean reduction in total daily symptoms scores from baseline. C) Mean doubling dilution (DD) difference in methacholine PC_{20} from baseline. D) Mean percentage reduction in fractional exhaled nitric oxide (FeNO) from baseline. All means have 95% confidence interval error bars. *Denotes significant within group change from baseline ($p<0.05$). Overweight group = closed circles and thick error bars. Normal weight group = open squares and thin error bars. ns = no significant difference.

Despite significant findings for FeNO and symptoms, there were no significant differences between overweight and normal groups for methacholine PC_{20} (Table 8; Figure 10, Plot C) although there were significant dose responses within both groups for this measure. There was a non-significant trend towards attenuated cortisol suppression in overweight subjects at 0-800ug budesonide (p=0.06) (Table 9). FEV_1 response (Table 10; Figure 10 Plot A) showed no significant differences between weight groups for change from baseline (0-200µg or 0-800µg), however, there was a significant difference between 200-800µg.

Table 8. Methacholine bronchial challenge.

	Overweight		**Normal Weight**		**Difference**	
	Mean (95%CI)	P	Mean (95%CI)	P	Mean (95%CI)	P
Baseline	0.80 (0.60, 1.07)*		0.61 (0.43, 0.87)*		1.30 (0.81, 2.09)	0.27
0-200µg	0.66 (0.33, 0.99)	<0.001	0.62 (0.26, 0.99)	0.003	0.04 (-0.48, 0.56)	0.88
0-800µg	1.16 (0.73, 1.59)	<0.001	1.11 (0.67, 1.56)	<0.001	0.05 (-0.62, 0.71)	0.89
200-800µg	0.50 (0.13, 0.87)	0.01	0.49 (0.06, 0.92)	0.03	0.01 (-0.59, 0.60)	0.98

Data are presented as geometric means* for baseline values (mg/ml) and mean doubling dilution differences (95% confidence intervals) in methacholine PC_{20} from baseline vs. 200µg (0-200µg ICS), baseline vs. 800µg (0-800µg ICS), 200µg vs. 800µg (200-800µg ICS), and between groups. For each variable p-values (P) are shown for both within group responses and also for the between group differences in response. Significance level $p<0.05$. ICS= Inhaled corticosteroid (HFA-budesonide).

Table 9. Overnight urinary cortisol/creatinine ratio (OUCC).

	Overweight		Normal Weight		Difference	
	Mean (95%CI)	P	Mean (95%CI)	P	Mean (95%CI)	P
Baseline	3.38 (2.72, 4.21)*		3.72 (2.80, 4.95)*		0.91 (0.63, 1.31)	0.60
0-200µg	1.02 (0.80, 1.31)	0.86	0.87 (0.63, 1.19)	0.40	1.17 (0.78, 1.76)	0.43
0-800µg	0.78 (0.63, 0.97)	0.03	0.53 (0.38, 0.75)	0.001	1.46 (0.98, 2.17)	0.06
200-800µg	0.76 (0.59, 0.99)	0.048	0.61 (0.39, 0.96)	0.04	1.24 (0.76, 2.03)	0.38

Data presented as geometric means (nmol/mmol)* for baseline values and geometric mean fold differences (95% confidence intervals) from baseline vs. 200µg (0-200µg ICS), baseline vs. 800µg (0-800µg ICS), 200µg vs. 800µg (200-800µg ICS), and between groups. For each variable p-values (P) are shown for both within group responses and also for the between group differences in response. Significance level $p<0.05$. ICS= Inhaled corticosteroid (HFA-budesonide).

Table 10. Forced expiratory volume in 1 second (FEV_1).

	Overweight		Normal Weight		Difference	
	Mean (95%CI)	P	Mean (95%CI)	P	Mean (95%CI)	P
Baseline	2.83 (2.66, 3.01)*		2.83 (2.54, 3.13)*		0.00 (-0.33, 0.32)	0.99
0-200µg	0.09 (0.03, 0.15)	0.004	0.03 (-0.04, 0.10)	0.40	0.06 (-0.04, 0.15)	0.22
0-800µg	0.07 (0.03, 0.11)	0.003	0.14 (0.07, 0.22)	0.001	-0.08 (-0.16, 0.01)	0.07
200-800µg	-0.02 (-0.06, 0.02)	0.37	0.11 (0.05, 0.18)	0.003	-0.13 (-0.21, -0.05)	0.001

Data are presented as arithmetic means* for baseline values and arithmetic mean differences (95% confidence intervals) for baseline vs. 200µg (0-200µg ICS), baseline vs. 800µg (0-800µg ICS), 200µg vs. 800µg (200-800µg ICS) and between groups. For each variable p-values (P) are shown for both within group responses and also for the between group differences in response. Significance level $p<0.05$. ICS= Inhaled corticosteroid (HFA-budesonide).

MANOVA comparisons of outcome variables demonstrated no significant interactions and confirmed there was an overall effect of BMI. Moreover there was no confounding effect of cumulative type 1 error from multiple T testing. Regression analyses of both FeNO and symptom responses at 0-200µg budesonide against BMI as a continuous variable show significant correlation and confirm the original between group findings. These are presented in Figure 11.

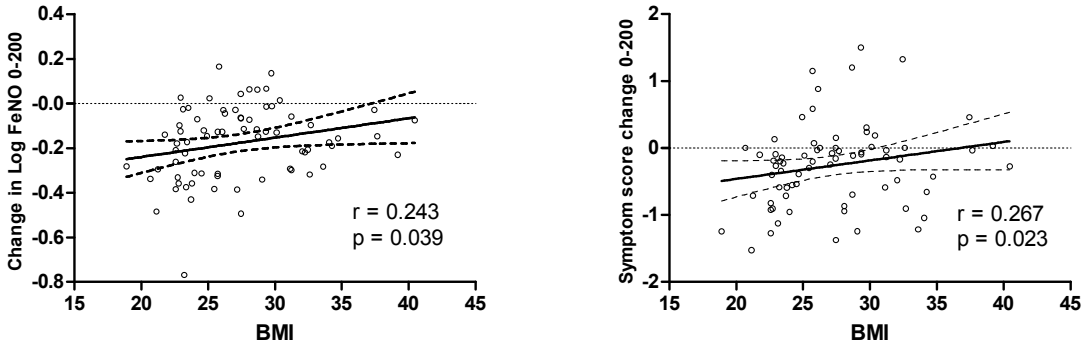

Figure 11. Regression analyses of change in Log FeNO (left) and symptom scores (right) for all patients between 0-200µg daily inhaled HFA budesonide against body mass index (BMI).

Interrupted lines represent 95% confidence intervals for the regression line of identity (solid line). r = correlation coefficient. P-value for the slope is also shown.

DISCUSSION

We have compared data on stepwise dose-response to ICS between normal weight and overweight persistent asthmatics. Based on previous work suggesting reduced responsiveness to ICS in overweight asthmatics at fixed doses, we used a variety of asthma outcome measures to assess for differences between weight groups. Unsurprisingly, the overweight group in our study were older than the normal group despite a non-significant correlation with BMI (continuous variable). This trend has been reported in previous studies examining obesity and asthma[231,237].

We found significantly greater improvement in total daily asthma symptom scores in the normal group, at both 0-200µg and 0-800µg ICS, compared to the overweight group. Most improvement was demonstrated between 0-200µg ICS. The lack of difference between groups in FEV_1 at either dose level may simply reflect that our patient cohort had relatively preserved baselines, with FEV_1 %predicted for normal and overweight groups 81.7% and 82.1% respectively, leaving little room for further improvement. In this regard it is well documented that in response to dose titration with ICS, effects on exacerbations are rather removed from pulmonary function[13,14]. Both baseline symptoms and FEV_1 were similar between groups, so cannot account for the disconnect we found between these outcome measures.

Altered lung mechanics have previously been theorised to explain lack of improvement in symptoms in overweight asthmatics, i.e., an independent reason rather than asthma causing symptoms. Indeed, excess adipose tissue, particularly in very obese patients, could reduce spirometric indices through external restriction, but we did not demonstrate this in our study. It has been previously shown that

overweight patients have a reduced functional residual capacity compared to normal weight patients leading to reduced airway calibre and increased airway resistance[239]. Increased elastic load on the airways has also been suggested, and found to be an independent predictor of dyspnoea during bronchial challenge[240]. The overweight group in our study had a mean BMI=30kg/m^2, which is not exceptionally high, with a difference of 7kg/m^2 between groups, hence it would be difficult to believe mechanical factors alone could explain the differences seen in symptoms. Indeed, a small change in FEV_1 (from the same baselines) was seen in both groups confirming that lung mechanics is probably not the major factor to explain the disconnect between FEV_1 and symptoms. It could be that symptoms are more closely related to airway inflammation and small airways function rather than large airway calibre, suggested by the connection we found between symptom and FeNO responses.

We also found a significantly greater reduction in FeNO in the normal weight group. This was again seen at both 0-200µg and 0-800µg, with most improvement again at 0-200µg. The normal group had a significantly higher baseline FeNO than the overweight group. This dose response difference could, therefore, be explained by a greater 'regression to the mean' in the normal group. However, the baseline FeNO difference was only significant when BMI was a categorical rather than a continuous variable. Previously it has been suggested that overweight asthmatics have a higher baseline FeNO[232] - the opposite to our cohort. FeNO is recognised as a surrogate measure of airway inflammation in asthma, reflecting airway eosinophilia[241]. The difference between weight groups on FeNO response was in contrast to the lack of any difference between groups in methacholine AHR. One might assume that AHR and FeNO should be highly correlated – i.e. the more inflammation in the airways, the

more 'twitchy' they should become to stimuli, hence response to ICS might be mirrored in both. Nonetheless, methacholine PC_{20} showed a shift from baseline within each group, which was significant even at the lower dose. There are conflicting findings for this assumption of correlation from previous studies directly comparing FeNO and methacholine[221,242]. Indeed, methacholine acts directly on airway smooth muscle, which may explain the disconnect between methacholine and FeNO in our study. One might speculate that 'indirect' challenges such as adenosine monophosphate (AMP), or perhaps mannitol, may have shown more correlation with FeNO response as these have effect through inflammatory mediators, then subsequently on airway smooth muscle. Alternatively, because methacholine acts on the central large airways, in contrast to FeNO, which reflects 'whole lung' inflammation, it alludes to the possibility of reduced peripheral lung deposition or absorption of ICS in overweight asthmatics. Further methods to examine this theory could include impulse oscillometry as a measure of peripheral airways function, or perhaps radiolabelled ICS peripheral lung deposition imaging studies between normal and overweight asthmatics.

It is postulated that obesity confers a 'pro-inflammatory' state by enhancing adipose tissue immune function via immune mediators including TNF-α, interleukin-6, leptin, adiponectin and resistin[243,244]. Moreover, there is a theory that this triggers up-regulation of pulmonary inflammatory mechanisms increasing levels of FeNO[232]. Unfortunately we did not measure any other inflammatory markers such as induced sputum eosinophils or extracellular cationic protein (ECP) to see if ICS response might also be blunted, like FeNO, in the overweight group. Another study has suggested that there may be reduced airway inflammation in overweight subjects as

assessed by eosinophil levels, also remarking that lesser control may be related to other external factors such as gastro-oesophageal reflux and sleep disordered breathing[245]. Our study suggests that it is not FeNO level per se that is important in overweight asthmatics, but that it may be a less sensitive outcome response to ICS compared to the normal group.

Our findings suggest that following ICS dose increments, both asthma symptoms and FeNO may improve more in normal compared to overweight asthmatics, with no difference in FEV_1 and methacholine response seen between groups. It may be that symptoms and FeNO are more subtle measures of improvement in the normal group with the lack of response in the overweight group supporting the theory of reduced peripheral lung deposition/absorption of ICS in overweight patients. We measured overnight urinary cortisol/creatinine ratio to reflect adrenal suppression in response to ICS, which correlates with systemic steroid absorption from the lung. Pointedly within both groups there was significant suppression at 0-800ug, along with a non-significant trend to attenuated adrenal suppression in the overweight group at 0-800µg with a 32% difference in cortisol response between groups (p=0.06). This in turn suggests reduced corticosteroid absorption in the overweight group and again the possibility of reduced peripheral lung deposition/absorption upstream. We did not however measure budesonide kinetics, so we cannot exclude the alternative explanation that the overweight group may have had reduced peripheral glucocorticoid responsiveness resulting in attenuated cortisol suppression. Indeed, the distribution of body fat may play a role here too where a prior study found that men with abdominal obesity showed less inhibition of serum cortisol by dexamethasone, particularly at lower concentrations, while in the normal weight control subjects,

cortisol secretion was inhibited in an apparent dose-response manner[246]. However, we have no data on distribution of body fat in this study. Inhaled budesonide has a relatively smaller volume of distribution than other more lipophilic inhaled corticosteroids such as fluticasone[247], making it an ideal inhaled steroid to reduce confounding between weight groups. Obesity has also been shown to alter response to glucocorticoids through MAP-kinase signalling pathways[236], however, Sutherland et al concedes that overall glucocorticoid insensitivity is likely to be complex, reflecting the multiple steps in glucocorticoid action.

One hypothesis we have generated from this study is that dose response to ICS may be blunted with regard to FeNO and symptoms in overweight asthmatics, particularly at lower doses. Peters-Golden demonstrated a markedly greater response of obese asthmatics to the leukotriene receptor antagonist montelukast as compared to ICS, ICS/LABA combination and placebo[237]. This is supported by the finding that leukotriene receptors are up-regulated by leptin, an adipocyte-derived hormone whose serum levels parallel total body fat mass[248,249]. It is worth considering that LTRAs could be added to ICS at an earlier stage in the stepwise therapy of overweight asthmatics, or indeed these patients may find greater benefit in terms of peripheral small airways disease using extra-fine particle ICS devices.

In conclusion, we hypothesise that overweight persistent asthmatics may have attenuated symptom and FeNO responses to stepwise ICS increments compared to normal weight asthmatic counterparts. Interestingly, we found no difference between weight groups in either FEV_1 or methacholine responses at either dose. There was a trend to attenuated cortisol suppression in overweight subjects, suggesting the

possibility of reduced bioavailability in this group. Further prospective studies are warranted to explore these findings, in particular whether there is reduced peripheral lung deposition/absorption of ICS in overweight asthmatics.

CHAPTER 5:

ADDING PROPRANOLOL TO LOW DOSE INHALED CORTICOSTEROID VERSUS HIGHER INHALED CORTICOSTEROID DOSING ALONE IN MILD-MODERATE PERSISTENT ASTHMA

Study aims:

1. To examine whether the inverse beta-2 receptor agonist propranolol (a non-cardioselective beta-blocker) provided any corticosteroid-sparing activity on histamine-induced airway hyperresponsiveness in persistent asthma.
2. To examine whether adding propranolol to low dose inhaled corticosteroid provided any additional anti-inflammatory effects in persistent asthma, compared to higher inhaled corticosteroid dosing.
3. To examine clinical safety outcomes when using propranolol in persistent asthmatics, in particular, effects on pulmonary function.

ABSTRACT

Background

The murine asthma model shows that switching off airway beta-2 receptors with an inverse agonist may confer anti-inflammatory effects as well as corticosteroid-sparing activity.

Objective

We assessed for any corticosteroid-sparing effects of propranolol, an inverse agonist, added to low dose inhaled corticosteroid (ICS) versus higher dose ICS.

Methods

A randomized, double-blind, placebo-controlled, crossover trial in mild-moderate persistent asthmatics was performed. After a run-in (2weeks) on hydrofluoroalkane-beclometasone dipropionate (HFA-BDP) 100µg/day, patients received randomised treatments (4weeks) with propranolol 80mg/day plus HFA-BDP 100µg/day; versus placebo plus HFA-BDP 400µg/day. Propranolol was up-titrated to 80mg/day over initial 2weeks. Tiotropium was co-administered until 5days before each histamine challenge (the primary outcome).

Results

16 patients completed the study, mean age 38yr; FEV_1 86.4%; histamine PC_{20} 1.39mg/ml; ICS dose 406µg/day. Histamine PC_{20} was unchanged by adding propranolol to HFA-BDP100 compared to baseline (HFA-BDP100): 0.17 doubling dilution (dd) difference (95%CI -0.58-0.92), but there was a significant improvement

with HFA-BDP400 compared to both baseline 1.05dd (95%CI 0.43-1.66), P=0.02; and propranolol + HFA-BDP100:0.88dd (95%CI 0.45-1.30), P=0.006. Significant improvements were also observed with HFA-BDP400 for exhaled nitric oxide, blood eosinophils, serum eosinophilic cationic protein and asthma quality of life questionnaire symptoms, compared to propranolol + HFA-BDP100. Salbutamol recovery post-challenge was partially blunted by propranolol (median prolongation 5min, P=0.002). Domiciliary evening FEV_1 also fell with propranolol +HFA-BDP100: mean reduction from baseline 0.22L [95%CI 0.10-0.34L], P=0.012, while Asthma Control Questionnaire remained unchanged.

Conclusion

The inverse agonist propranolol produced no improvements when given with low dose ICS, while further significant improvements in airway hyper-responsiveness and inflammation were demonstrated with higher dose ICS. Thus propranolol does not confer corticosteroid-sparing activity in persistent asthma.

Ethics Reference: 11/ES/0031

EudraCT number: 2011-002512-89

MHRA reference: 21726/0278/001-0001

ClinicalTrials.gov reference: NCT01544634

INTRODUCTION

Current asthma treatment advocates a step-wise approach, with inhaled corticosteroids (ICS) the main anti-inflammatory controller therapy and long-acting beta-2 agonists (LABA) the preferred second-line bronchodilator add-on therapy[11]. However, despite optimizing ICS/LABA, many patients remain poorly controlled[250]. Inevitably some patients require higher ICS dosing, highlighting the unmet need for new ICS-sparing strategies.

One would not expect any novel asthma therapy to come in the guise of a drug long-considered hazardous in asthmatics due to the risk of profound acute bronchoconstriction[251]; nevertheless beta-blockers have been the subject of recent interest and study in this regard. Murine asthma models demonstrated improvements in airway hyper-responsiveness (AHR)[252], up-regulation of beta-2 adrenoceptors (B2ADRs)[252,253], and a reduction in airway inflammation following chronic beta-blockade with nadolol[254]. Moreover, in humans, open-label chronic nadolol therapy in steroid-naïve asthmatics[255,256] resulted in significantly improved AHR, achieving a 1.8 doubling dilution (dd) increase in methacholine PC_{20} (provocative concentration causing 20% FEV_1 fall), almost twice the minimum important difference[257].

Regarding the pivotal safety issue of using beta-blockers in asthma, there was no adverse signal from a meta-analysis of chronic beta-blocker studies in patients with reactive airways disease for FEV_1, symptoms or reliever use[258]. Prospective open-label studies by Hanania et al[256] and Short et al[259] have shown that the response to high-dose salbutamol rescue was only partially blunted post-bronchial challenge in

asthmatics receiving either chronic nadolol or single-dose propranolol respectively, with only minor FEV_1 reductions.

We recently published data showing no additional therapeutic benefit of chronic propranolol versus placebo when added to medium-dose ICS on AHR to either histamine or methacholine[4]. This was surprising given the marked effect seen in ICS-naïve patients using nadolol; however, this could perhaps be explained by the fact that ICS themselves act through both up-regulation of airway B2ADRs[89] as well as being first-line anti-inflammatory treatment for asthma, therefore, leaving no room for further improvement.

The next logical question, therefore, is whether there may be any corticosteroid-sparing effect of chronic beta-blockade on AHR and other inflammatory outcomes in asthma. This hypothesis is supported by data showing steroid-sparing properties of nadolol using the mouse asthma model[260]. In this study we wished to examine whether chronic propranolol treatment added to low dose ICS, was non-inferior to increasing the dose of ICS alone on the primary outcome of histamine AHR in persistent asthmatics; i.e. specifically testing for a corticosteroid-sparing effect.

METHODS

Patients

Stable, histamine responsive (PC_{20}<8mg/ml) persistent asthmatics receiving ≤1000μg/day of ICS (beclometasone dipropionate equivalent), (CONSORT diagram Figure 12). The main exclusion criteria were: uncontrolled asthma symptoms; FEV_1<60% predicted; resting systolic blood pressure (BP) <110mmHg; resting heart rate (HR) <60bpm; any degree of heart block assessed by electrocardiogram; current use of negatively chronotropic medication; asthma exacerbation requiring oral steroid use in previous 6 months; and pregnancy.

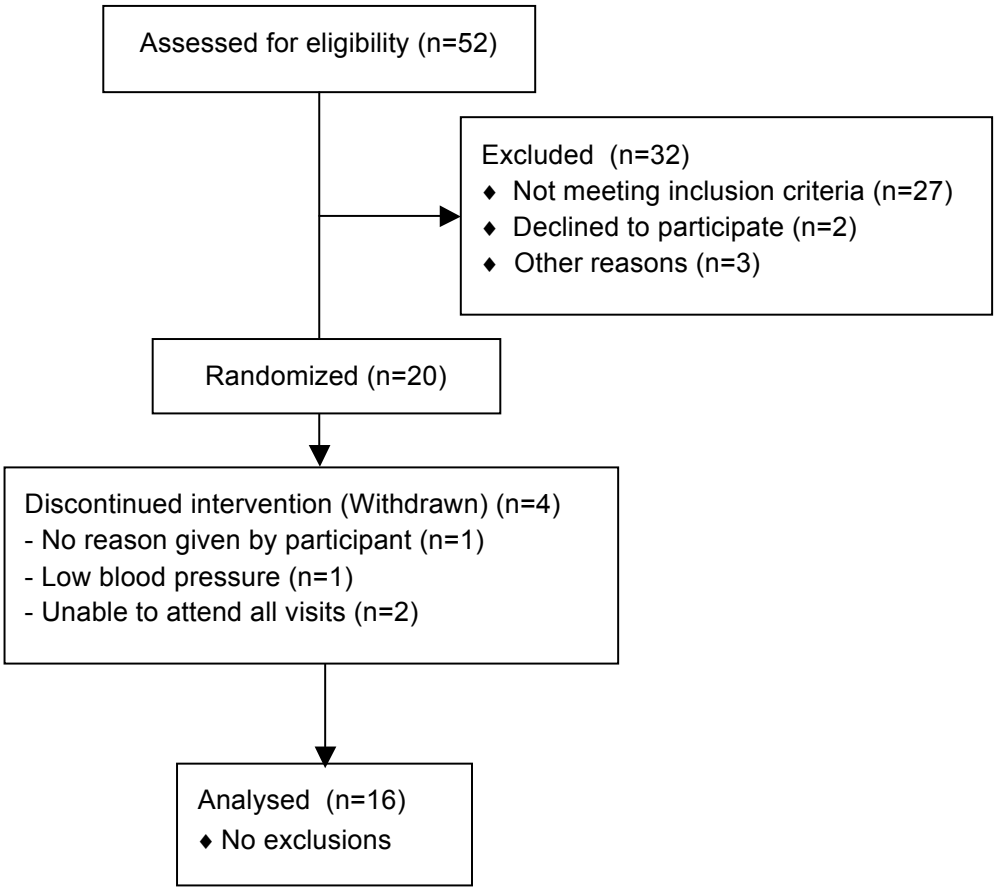

Figure 12. CONSORT diagram of patient recruitment.

Design

Single-centre, randomized, double-blind, placebo-controlled, crossover study. Following a two-week run-in with hydrofluoroalkane-beclometasone dipropionate (HFA-BDP, Qvar® Easibreathe, TEVA Pharmaceuticals, Horsham, USA) 100µg/day, ex-valve dose (80µg ex-actuator), patients were randomized to either: A) HFA-BDP 100µg/day plus propranolol sequentially up-titrated from 10mg bid (one week), to 20mg bid (one week), then 80mg (sustained release) od (two weeks); B) HFA-BDP 400µg/day, ex-valve dose (320µg ex-actuator), plus matched placebo for four weeks (Figure 13). Participants commenced tiotropium 18µg od (Spiriva® Handihaler, Boehringer Ingelheim Ltd, Berks, UK) at the beginning of run-in and both treatments, to mitigate against potential bronchoconstriction upon initial propranolol exposure[261]; then discontinued five days prior to each histamine challenge.

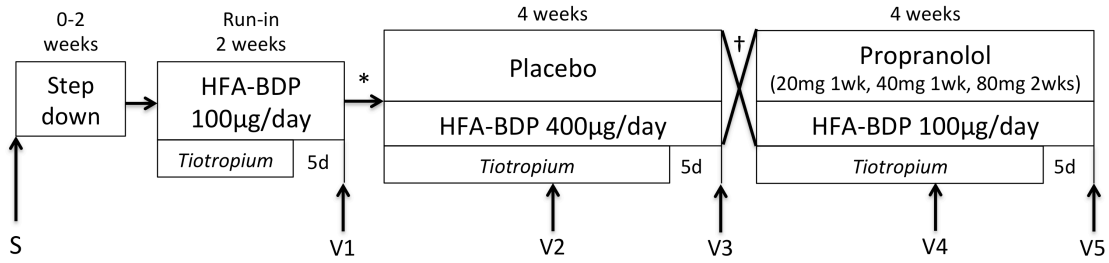

Figure 13. Study flow diagram.

Screening visit (S). Tiotropium stopped 5 days (5d) prior to histamine challenges at main study visits 1, 3 and 5 (V1, V3, V5). Patients randomized (*) at V1. Cross over (†) to remaining treatment arm. Safety visits at 2 weeks into treatment periods (V2, V4). Study complete at end of V5.

Patients were monitored in our department for two hours following administration of the first dose of propranolol (or placebo) at visits one and three; with safety

measurements (HR, BP, FEV_1) assessed prior to up-titration to 80mg propranolol (or placebo) at visits two and four. At the end of the first randomised treatment arm, patients were directly crossed over to the remaining treatment arm.

Alongside initial ICS step-down, patients withheld any concomitant LABA or other third-line asthma therapy including antihistamines for 1 week prior to run-in and for the duration of the study.

The primary outcome was histamine PC_{20} comparing post run-in baseline with completed treatment periods. Secondary outcome measures were: spirometry; impulse oscillometry (IOS); exhaled nitric oxide (FeNO); blood eosinophils; serum eosinophilic cationic protein (ECP); overnight urinary cortisol/creatinine ratio (OUCC); pre/post-histamine challenge measurements (HR, BP, serum potassium, FEV_1); domiciliary FEV_1; Asthma Quality of Life (AQLQ) and Asthma Control Questionnaires (ACQ).

St Mary's Pharmaceutical Unit, Cardiff, Wales, UK performed blinding and randomisation of investigational medicinal products.

Measurements

Histamine was dispensed via nebulized solution (Tayside Pharmaceuticals, Dundee, UK), and the challenge was performed in accordance with standard guidelines. A dosimeter (Markos-Mefar, Bovezzo (BS), Italy) was used with doubling concentrations of histamine from 0.3125mg/ml-40mg/ml. The PC_{20} was then calculated by computer assisted log linear interpolation of the dose response.

Nebulized albuterol (5mg) was used to recover patients immediately post challenge. FEV_1 was measured every 5mins for 30mins post challenge or until it returned to within 5% of baseline. If FEV_1 had not recovered by 15mins, nebulized ipratropium bromide (500µg) was additionally administered. HR and serum potassium were measured pre-challenge and 15mins post albuterol administration. All HR and BP measurements were recorded in triplicate. Exhaled tidal nitric oxide was measured in line with ATS recommendations[22] using a NIOX analyzer (NIOX® Nitric Oxide Monitoring System, Aerocrine AB, Solna, Sweden) prior to other pulmonary function measures. Impulse oscillometry (Masterscreen IOS, Höchberg, Germany) was performed in accordance with manufacturer's guidelines. Spirometry was performed using a SuperSpiro (Carefusion, Basingstoke, UK) according to ATS guidelines[123]. Domiciliary FEV_1 was performed in triplicate both morning and evening using a handheld PiKO monitor (n-Spire Health, Longmont, CO, USA) according to manufacturer's instructions. Peripheral blood eosinophils were measured using the Sysmex XE2100 Hematology autoanalyser. Serum ECP was measured in duplicate using a UniCAP system (Phadia, Milton Keynes, UK) - coefficient of variation 3%. Overnight urine was collected between 22.00h and 08.00h. Urinary free cortisol was measured using a commercial radioimmunoassay kit (DiaSorin Ltd., Wokingham, Berkshire, UK). The intraassay coefficient of variation (Cv) was 21%, with the interassay Cv 13%. Urinary creatinine was measured using the RATE technique on a Cobas-Bio auto analyzer (Roche Products, Welwyn Garden City, UK) with an interassay Cv of 4.3%. Skin prick testing was performed using standard allergen extracts (Diagenics Ltd, Milton Keynes, UK).

Statistical Analyses

All data were assessed for normality of distribution. Non-normally distributed variables were logarithmically transformed or analyzed non-parametrically. The null hypothesis was that compared to post run-in baseline on HFA-BDP 100µg/day, adding propranolol 80mg/day confers no change to Histamine PC_{20}, whereas increasing the dose of HFA-BDP alone (400µg/day) causes a significant improvement (positive control). To detect a minimal important difference of 1dd in histamine PC_{20} between randomized treatments, assuming a within-subject standard deviation of 1.3dd, required 16 participants to achieve 80% power with an alpha-error of 0.05 (two-tailed) using a crossover design (matched subjects). Analysis was performed using repeated measures analysis of variance, factoring treatment and sequence effects, followed by Bonferroni correction for all pairwise comparisons ($P<0.05$, two-tailed). All analyses were performed using IBM SPSS v20 (San Diego, CA, USA).

RESULTS

Participants

16 completed per protocol (Table 11, Figure 12). Mean FEV_1 was 86.4% predicted (95%CI: 80.9, 91.9) and the geometric mean histamine PC_{20} was 1.39mg/ml (95%CI: 0.93, 2.07) at screening. There were no significant adverse events.

Table 11. Demographics.

Variable	N=16
Age (yr)	38 (31, 44)
Gender (M:F)	6:10
BMI (kg/m^2)	26.9 (24.6, 29.2)
Atopy (>1 SPT positive; n)	14
SPT positivity*	2.5 (1, 4)
BDP ICS (µg/day)	406 (243, 569)
LABA (all combined with ICS; n)	7
Antihistamine / LTRA (n)	2 / 2
FEV_1 (% predicted)	86.4 (80.9, 91.9)
FEF_{25-75} (% predicted)	53.6 (46.5, 60.7)
R5 (% predicted)	151 (126, 181)
R5-R20 (kPa/L/sec)	0.13 (0.07, 0.19)
FeNO (ppb)†	40 (29, 55)
Histamine PC_{20} (mg/ml)†	1.39 (0.93, 2.07)
Systolic BP (mmHg)	131 (122, 140)
Diastolic BP (mmHg)	79 (73, 84)
HR (bpm)	73 (68, 78)

Data presented as mean (95% confidence intervals [CI]) or absolute values unless stated. *Median (interquartile range). †Geometric mean (95%CI). BMI = body mass index. SPT = skin prick test to allergen. BDP ICS = beclometasone dipropionate equivalent inhaled corticosteroid dose. LABA = long-acting beta-2 agonist. LTRA = leukotriene receptor antagonist. FEV_1 = forced expiratory volume in 1s. FEF_{25-75} = forced expiratory flow between 25-75% of forced vital capacity. R5 = resistance at 5Hz (total airway resistance) from impulse oscillometry. R5-R20 = resistance between 5Hz and 20Hz (peripheral airway resistance). FeNO = fractional exhaled tidal nitric oxide. PC_{20} = provocative concentration (of histamine) causing 20% fall in FEV_1. BP = blood pressure. HR = heart rate. Ppb = parts per billion. Bpm = beats per minute.

Airway hyper-responsiveness

The primary outcome, histamine PC_{20}, showed no significant difference adding propranolol to low-dose HFA-BDP 100µg/day versus post run-in baseline on HFA-BDP 100µg/day alone (Table 12, Figure 14a): mean difference 0.17dd (95%CI: -0.58, 0.92). There was, however, a significant improvement in PC_{20} with higher dose HFA-BDP 400µg/day and placebo versus both baseline, 1.05dd (95%CI: 0.43, 1.66), P=0.02, and propranolol, 0.88dd (95%CI: 0.45, 1.30), P=0.006 (Figure 15).

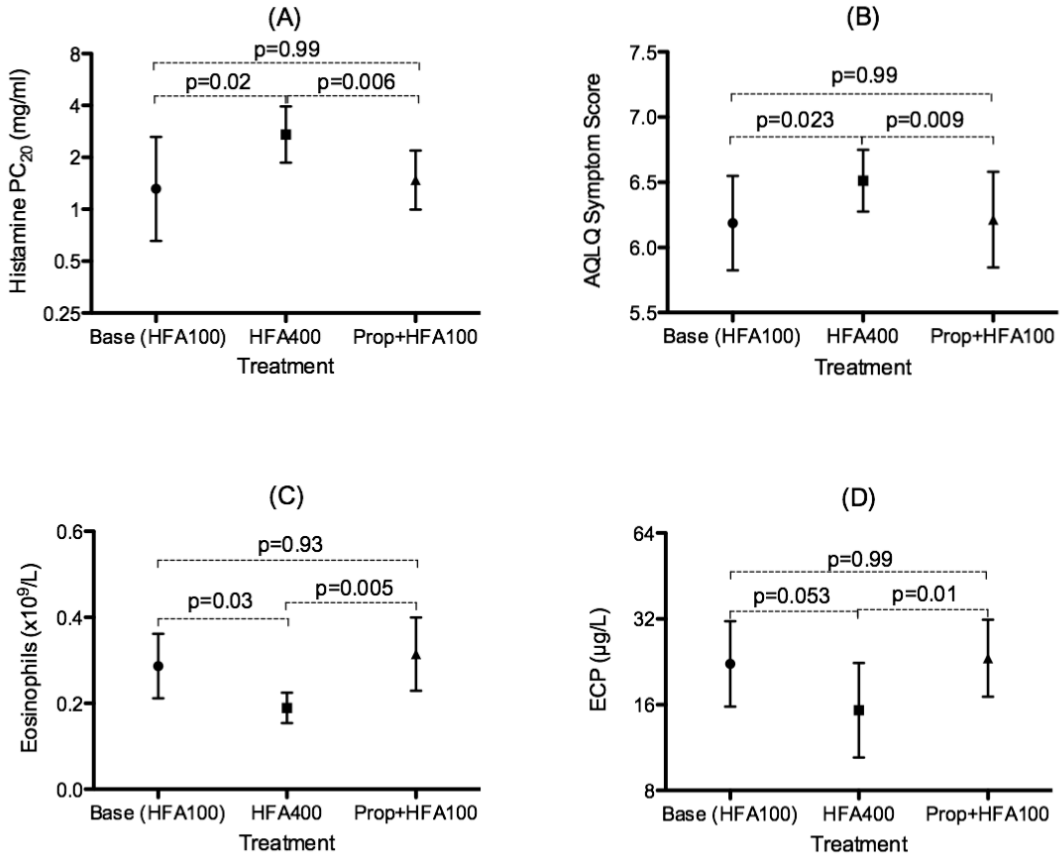

Figure 14. Airway hyper-responsiveness, symptoms, blood eosinophils and serum ECP.

Data presented as arithmetic means (unless stated) with 95% confidence interval (CI) error bars for baseline on hydrofluoroalkane beclometasone dipropionate (HFA) 100µg/day (circle), HFA 400µg/day plus placebo (square), and propranolol 80mg/day plus HFA 100µg/day (triangle). (A) Histamine PC_{20} (primary outcome). Geometric means (95%CI). (B) Asthma Quality of Life Questionnaire (AQLQ) symptom component. (C) Blood eosinophils. (D) Serum eosinophilic cationic protein (ECP). Geometric means (95%CI).

Table 12. Airway hyper-responsiveness; local and systemic inflammation; pulmonary function; adrenal function; asthma control; and cardiovascular outcomes.

Variable	Baseline HFA-BDP 100 (†)	HFA-BDP 400 + Placebo (‡)	HFA-BDP 100 + Propranolol (§)	P-value (ANOVA)
Histamine PC_{20} (mg/ml)*	1.31 (0.66, 2.63)	2.71 (1.87, 3.95)†,§	1.48 (1.00, 2.19)	0.007
FeNO (ppb)*	29 (21, 41)	19 (15, 25)†	25 (18, 35)	0.01
Eosinophils ($x10^9$/L)	0.29 (0.22, 0.35)	0.19 (0.16, 0.22)†,§	0.31 (0.24, 0.39)	0.001
ECP (µg/L)*	22.3 (15.8, 31.4)	15.3 (10.5, 22.4)§	23.3 (17.1, 31.8)	0.006
OUCC (nmol/mmol)*	5.14 (3.92, 6.75)	3.85 (2.71, 5.46)	3.73 (2.65, 5.27)	0.22
FEV_1 (% Pred)	91.6 (86.6, 96.7)	91.4 (87.1, 95.6)	88.9 (84.9, 92.9)	0.21
FEF_{25-75} (% Pred)	60.3 (50.6, 69.9)	58.8 (50.3, 67.3)	54.6 (47.6, 61.7)	0.055
R5 (% Pred)*	135 (111, 165)	144 (123, 169)	152 (130, 178)	0.22
R5-R20 (kPa/L/s)*	0.06 (0.03, 0.12)	0.08 (0.05, 0.13)	0.10 (0.06, 0.16)	0.21
ACQ score	0.57 (0.30, 0.84)	0.49 (0.29, 0.69)	0.65 (0.36, 0.94)	0.41
AQLQ symptoms	6.19 (5.87, 6.51)	6.51 (6.30, 6.72)†,§	6.21 (5.89, 6.54)	0.004
AQLQ overall	6.16 (5.88, 6.44)	6.41 (6.15, 6.67)	6.20 (5.92, 6.49)	0.053
Supine HR (bpm)	75.3 (70.5, 80.0)	76.1 (71.7, 80.5)	65.7 (62.2, 69.3)†,‡	<0.001
Supine Systolic BP (mmHg)	127 (119, 135)	127 (118, 135)	119 (112, 126)†,‡	0.01
Supine Diastolic BP (mmHg)	79 (73, 85)	79 (73, 85)	71 (66, 77)†,‡	<0.001

All data presented as mean values (95% confidence intervals [CI]) unless stated. *Geometric mean (95%CI). Overall significance with repeated measures analysis of variance (ANOVA). Significant ($P<0.05$) pairwise comparisons using Bonferroni correction are displayed as †, ‡ or § in superscript where the value is significantly different to respective named columns. HFA-BDP = hydrofluoroalkane beclometasone dipropionate. PC_{20} = provocative concentration causing 20% fall in FEV_1. FeNO = fractional exhaled tidal nitric oxide. Ppb = parts per billion. ECP = serum eosinophilic cationic protein. OUCC = overnight urinary cortisol/creatinine ratio. FEV_1 = forced expiratory volume in 1s. FEF_{25-75} = forced expiratory flow between 25-75% of forced vital capacity. R5 = resistance at 5Hz. R5-R20 = difference between resistance at 5Hz to 20Hz. ACQ = Asthma Control Questionnaire. AQLQ = mini Asthma Quality of Life Questionnaire. %Pred = percentage of predicted. HR = heart rate. Bpm = beats per minute. BP = blood pressure.

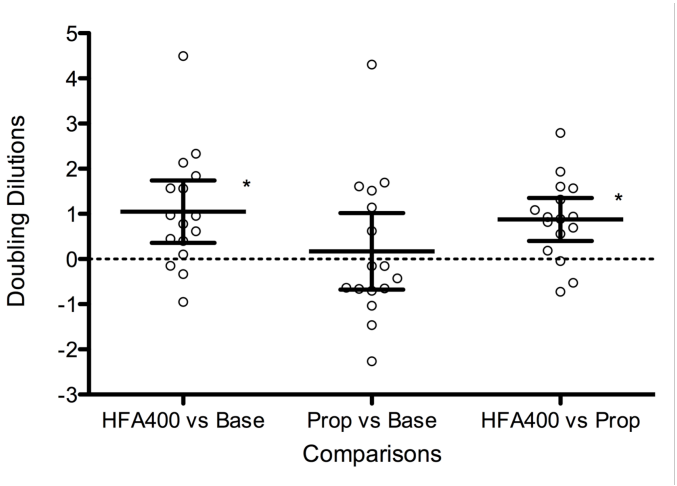

Figure 15. Histamine PC_{20} doubling dilution differences.

Scatter plots of individual histamine doubling dilution differences between all comparisons of baseline and both treatments with mean and 95% confidence interval error bars. Horizontal interrupted line indicates unity. *P<0.05 between treatments. Base = baseline measurements post run-in on hydrofluoroalkane beclometasone dipropionate (HFA) 100µg/day. HFA400 = 400µg/day plus placebo. Prop = propranolol plus HFA 100µg/day.

Cardiovascular effects and salbutamol recovery

Propranolol produced significant effects on other outcomes: 80mg/day caused significant reductions from baseline in both supine HR by approximately 10bpm, P<0.001 and supine BP after chronic dosing (Table 12). Furthermore, recovery time to within 5% of baseline FEV_1 post- salbutamol was significantly blunted by propranolol, compared to both baseline and placebo (median prolongation 5min), P=0.002 (Table 13, Figure 16a), as was post-recovery FEV_1 - mean reduction 6.4% (95%CI: 3.7, 9.1%) from baseline, P=0.001 (Table 13, Figure 16d). Three patients on propranolol required ipratropium bromide 15mins post-salbutamol to fully recover post-challenge, compared with no patients following baseline or placebo challenges, P=0.05. All active propranolol patients had recovered by 25mins post-salbutamol. There was no salbutamol-induced fall in serum potassium post-challenge with propranolol, compared to significant reductions after baseline, P<0.001, and placebo,

P=0.002, challenges (Table 13, Figure 16c). Similar significant blunting of response was seen in post-challenge HR and BP measurements with propranolol (Table 13, Figure 16b), compared to baseline and placebo.

Table 13. Salbutamol recovery outcomes post-histamine challenge.

Variable	Baseline HFA-BDP 100 (†)	HFA-BDP 400 + Placebo (‡)	HFA-BDP 100 + Propranolol	P-value (ANOVA)
FEV_1(% Pred)	92.8 (88.4, 97.1)	91.7 (87.9, 95.5)	86.4 (82.3, 90.4)†,‡	0.001
Time to >95% base FEV_1 (min)*	5 (5, 5)	5 (5, 10)	12.5 (5, 15)	0.002§
IB given at 15min (n)**	0	0	3	0.05††
Supine HR(bpm)	93.3 (87.0, 99.5)	96.0 (92.1, 99.9)	65 (60, 71)†,‡	<0.001
Supine Systolic BP (mmHg)	135 (128, 143)	136 (129, 143)	122 (114, 129)†,‡	<0.001
Potassium (mmol/L)	3.6 (3.4, 3.8)	3.6 (3.4, 3.8)	4.3 (4.1, 4.4)†,‡	<0.001

FEV_1 and IB data are following recovery to within 5% of baseline FEV_1 monitored at 5min intervals. HR, BP and Potassium were measured at 15min post nebulized salbutamol 5mg. All data presented as mean values (95% confidence intervals [CI]) unless stated. *Median (Interquartile range). **Absolute number of cases. Overall significance with repeated measures ANOVA unless stated. Significant ($P<0.05$) pairwise comparisons using Bonferroni correction are displayed as † or ‡ in superscript where the value is significantly different to respective named columns. §Related samples Friedman's two-way ANOVA. ††Related samples Cochran's Q test. HFA-BDP = Hydrofluoroalkane beclometasone dipropionate. FEV_1 = forced expiratory volume in 1s. IB = ipratropium bromide. HR = heart rate. Bpm = beats per minute. BP = blood pressure.

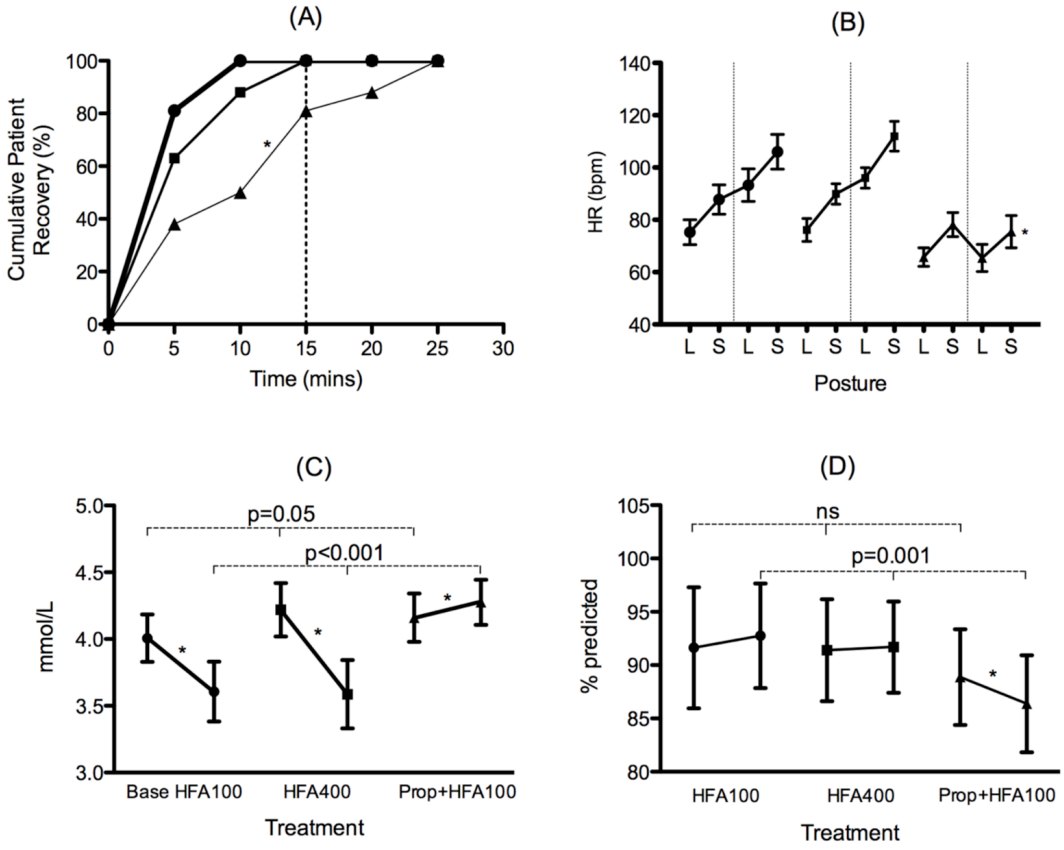

Figure 16. FEV_1 recovery post-salbutamol; heart rate, serum potassium, and FEV_1 pre and post-salbutamol.

Arithmetic means (unless stated) with 95%CI: baseline HFA100µg/day (circle), HFA400µg/day plus placebo (square), and propranolol 80mg/day plus HFA100µg/day (triangle). (A) Cumulative recovered patients >95% baseline FEV_1. Data: absolute values. Vertical line: ipratropium administration. *P=0.02 propranolol versus baseline/placebo. (B) Heart rate. L/S=lying/standing. Vertical lines: pre/post-challenge. *P<0.001 propranolol versus baseline/placebo. (C) Potassium. Lines join pre/post-challenge (*P<0.05). (D) FEV_1. Lines join pre/post-challenge (*P<0.05).

Inflammation

We also assessed local and systemic inflammatory outcomes – FeNO, blood eosinophilia and serum ECP - finding no significant difference in any outcome with propranolol compared to baseline. However, there were significant reductions with high dose ICS plus placebo versus baseline for both FeNO (Table 12), P=0.02, and

eosinophil levels, P=0.03 (Table 12, Figure 14c). Serum ECP was significantly lower with high dose ICS plus placebo versus propranolol, P=0.01 (Table 12, Figure 14d).

Symptoms and asthma control

Propranolol did not cause any significant change in AQLQ or ACQ over baseline values (Table 12). High dose ICS did lead to significant improvements in AQLQ symptom component score versus both baseline and propranolol, but less than the minimal important difference of 0.5 (Table 12, Figure 14b).

Airway caliber

There was a significant reduction in domiciliary evening FEV_1 with propranolol versus both baseline and placebo - mean 0.22L (95%CI: 0.10, 0.34) from baseline, P=0.012. This was only evident with the highest propranolol dose (80mg/day) and not with 40mg/day during week 2 of up-titration (Table 14). There was no significant fall in domiciliary morning FEV_1 with either propranolol dose (Table 14). Visit-based spirometry and IOS measures, including total airway resistance at 5Hz (R5), did not significantly change with either randomized treatment versus baseline (Table 12).

Adrenal function

We found no significant systemic suppression of adrenal function, using OUCC, with either treatment versus baseline (Table 12).

Table 14. Domiciliary FEV_1 at mid-point and end of treatments.

Variable	Baseline HFA-BDP 100 (a)	HFA-BDP 400 + Placebo (b)	HFA-BDP 100 + Propranolol (c)	P-value (ANOVA)
Propranolol 40mg				
Morning FEV_1 (L)	2.96 (2.60, 3.33)	2.99 (2.67, 3.30)	2.91 (2.58, 3.24)	0.32
Evening FEV_1 (L)	2.98 (2.62, 3.34)	2.95 (2.65, 3.26)	2.90 (2.57, 3.22)	0.19
Propranolol 80mg				
Morning FEV_1 (L)	2.96 (2.60, 3.33)	2.95 (2.65, 3.26)	2.84 (2.53, 3.15)	0.08
Evening FEV_1 (L)	2.98 (2.62, 3.34)	2.92 (2.60, 3.24)	2.76 (2.46, 3.06)[a,b]	0.002

Domiciliary data presented as 7-day rolling mean (95%CI) values over week 2 of run-in as well as week 2 (propranolol 40mg/day or placebo) and week 4 (propranolol 80mg/day or placebo) of both treatment periods. Overall significance with repeated measures analysis of variance (ANOVA). Significant ($P<0.05$) pairwise comparisons using Bonferroni correction are displayed as *a, b or c* in superscript where the value is significantly different to respective named columns. FEV_1 = forced expiratory volume in 1s. HFA-BDP = hydrofluoroalkane beclometasone dipropionate.

DISCUSSION

The results of the present study demonstrated that adding propranolol to low dose ICS in persistent asthma did not significantly change histamine AHR, whilst increasing the dose of ICS conferred a significant further improvement. In other words, propranolol did not demonstrate any corticosteroid-sparing effect on AHR, a hallmark of the underlying asthmatic disease process. Moreover, we found significant room for further improvement with the higher ICS dose for local and systemic inflammatory outcomes, namely FeNO, blood eosinophils and serum ECP, while adding propranolol to low dose ICS did not significantly impact on these.

The novel counterintuitive theory that beta-blockade might be effective in the treatment of asthma came through beta-adrenoceptor basic science and murine models of asthma. Inverse agonist activity occurs at the B2ADR in the presence of certain beta-blockers including propranolol and nadolol, essentially 'switching off' the receptor by reducing basal constitutional activity[262,263] with the potential, therefore, to up-regulate other B2ADRs. However other data would tend to negate the role of inverse agonism, whereby epinephrine depletion in the mouse model resulted in inhibition of allergen-induced asthma[61]. Other theories have postulated that certain beta-blockers such as nadolol and timolol might confer anti-asthmatic properties unrelated to their inverse agonism, such as inhibition of pro-inflammatory signaling pathways involving beta-arrestin and extracellular signal regulated kinases (ERK)[263,264]. Indeed, adrenaline depleted mice again demonstrated that any anti-inflammatory effect might be ligand specific, where the asthma phenotype developed in the presence of propranolol but not nadolol in these mice; and in the wild type mice propranolol did not prevent the occurrence of asthma, where nadolol was

preventative[265]. However the putative role of such in vitro anti-inflammatory activity in human asthmatics remains to be proven in placebo-controlled trials.

Open-label studies in mild steroid-naïve asthmatics showed that nadolol resulted in a 4.7% fall in pre-challenge FEV_1, with a preserved bronchodilator response to high dose salbutamol post-challenge[255,256]. This preserved salbutamol response might be explained by previous data from human peripheral blood lymphocytes which have shown that marked B2ADR up-regulation occurs within 2 days of chronic exposure to propranolol, with a fully restored isoproterenol-induced cyclic-AMP response after 2 weeks[266]. In our previous study[4] and in the current report we have shown partial blunting of salbutamol recovery post-challenge with propranolol versus placebo. Pointedly, the partially preserved bronchodilator response to salbutamol post-challenge in the presence of propranolol occurred despite complete attenuation of systemic B2ADRs in the form of salbutamol-induced hypokalemic and chronotropic responses, in turn suggesting much higher local than systemic receptor occupancy by inhaled salbutamol. Presumably it might be possible to completely overcome airway B2ADR occupancy by propranolol if one was to give much higher nebulized doses of salbutamol than the 5mg used in the present study. We were cautious about doing this because of the possibility of causing greater B2ADR mediated systemic adverse effects in the placebo arm.

Studies have consistently shown a dose response to ICS for AHR in asthma[267]. This was integral to our study design by including a positive control arm with high dose ICS, both to demonstrate any room for further improvement over low dose ICS and therefore any putative steroid-sparing effect of propranolol on AHR, as well as to

confirm that we had adequate power to detect any clinically important change in the primary outcome of histamine AHR. It is clear, therefore, from this study that there is no effect of chronic propranolol on AHR in persistent asthma as the histamine PC_{20} values were similar both pre- and post-propranolol, while taking the same 100µg dose of HFA-BDP. This adds to our previous data, where we found no additional effect of chronic propranolol over and above medium dose ICS at a BDP equivalent dose of 440µg/day[4], by answering the question of whether there might have been no additional room for improvement in AHR at this dose.

Dose-dependent ICS effects have also been demonstrated on other inflammatory outcomes. There is a significant dose response on FeNO with incremental ICS[1,159], albeit most improvement occurs with low dose ICS. Nevertheless, we did demonstrate significant improvements in FeNO, blood eosinophilia and serum ECP with the increased dose of ICS, but not with propranolol, suggesting there is no additional anti-inflammatory effect with chronic beta-blockade in the presence of low dose ICS. Furthermore, given that we found no difference between randomized treatments in regard to urinary cortisol levels, these effects on systemic allergic biomarkers (eosinophils and ECP) are more likely to be due to topical effects on the airway of higher dose HFA-BDP rather than any increased systemic activity.

We believe it noteworthy that chronic propranolol did not result in any significant worsening of AHR, FeNO, eosinophils, ECP, symptoms (AQLQ) or overall asthma control (ACQ); with only a minor reduction in domiciliary evening FEV_1. Indeed this was with a usual 80mg dose of sustained-release propranolol sufficient to cause a 10bpm mean reduction in supine HR as well as a significant reduction in resting BP.

On the basis of the screening FEV_1 (mean 86.4%), ICS dose (406ug/day), AHR (mean PC_{20} 1.39mg/ml), and FeNO levels (mean 40ppb), our cohort would be classified as having persistent asthma; one would therefore have expected them to fare worse when given propranolol especially while only taking low dose ICS. This preservation of outcomes on propranolol may perhaps be due to using a gradual dose ramp[255] of propranolol over several weeks, as well as co-prescribing the long-acting muscarinic antagonist tiotropium in order to avert any potential catastrophic fall in FEV_1 upon first exposure to beta-blockade[261]. However, it may also simply be that the presence of ICS even at low dose is intrinsically bronchoprotective against propranolol.

In keeping with previous studies that found significant reductions in FEV_1 (approximately 5%) with chronic beta-blockade[255,259], we also demonstrated a significant reduction in evening domiciliary FEV_1 with propranolol amounting to 0.22L (i.e. a 7.4% fall). Furthermore, we did not demonstrate any significant improvement with high dose ICS on airway caliber measurements, presumably because these were relatively preserved at baseline. There was a small improvement in the AQLQ symptom score with high dose ICS, but this was less than the minimum important difference of 0.5[35]. Moreover there was no change in ACQ throughout the study, with mean scores indicating well-controlled asthma from the outset.

We believe the strengths of our study are in its prospective, randomized, placebo-controlled design, particularly with regard to including a positive control arm (high dose ICS), which proved effective in demonstrating potential room for further improvement in the primary outcome measure of histamine AHR. Furthermore, we have shown that this study design has robust safety attributes in utilizing a gradual

dose ramp for propranolol, and providing protection with concomitant long-acting muscarinic antagonist therapy during this up-titration period.

One of the main limitations of our study may also be related to our focus on safety as evidenced by initial clinical stability (as seen by ACQ score) and relatively well preserved pulmonary function on study entry, thus leaving relatively little room for improvement in these outcomes upon chronic propranolol exposure. Another limitation may be due to small patient numbers, although the study was powered on the primary outcome of histamine AHR in terms of being able to detect a difference of one doubling dilution. It could also be reasonably argued that we should have employed nadolol as the beta-blocker of choice as used in the previous studies of nadolol in steroid naïve asthmatics. However, due to the available dosage of nadolol in the UK, the cost of formulating specific doses of nadolol at great expense, as well as the fact that the prevailing theory at the time of writing this study protocol was that of inverse agonism (which both nadolol and propranolol display), we deemed it plausible that propranolol would be a suitable substitute for nadolol.

In conclusion, adding propranolol to low dose ICS in persistent asthma provides no corticosteroid-sparing effect on histamine AHR and other inflammatory outcomes, compared to the significant improvements conferred by using a higher ICS dose. Salbutamol recovery post-histamine challenge was partially blunted and there was a small but significant fall in evening FEV_1 when adding propranolol to low dose ICS, but no deterioration in ACQ or AQLQ. One would not therefore advocate propranolol as a corticosteroid-sparing agent in persistent asthma. However, clinical equipoise might still be challenged as propranolol could perhaps be given at these

doses to asthmatic patients for its usual indications provided our strict safety criteria are adhered to in carefully selected patients controlled on ICS.

CHAPTER 6:

RELATIONSHIPS OF MANNITOL CHALLENGE TO METHACHOLINE CHALLENGE AND INFLAMMATORY MARKERS IN PERSISTENT ASTHMATICS RECEIVING INHALED CORTICOSTEROIDS.

Study Aims:

1. To examine the relationships of putative categorical cut-off values for mannitol airway hyperresponsiveness (AHR) severity against previously described severity cut-off values for methacholine AHR, and severity of airway inflammation (as measured by fractional exhaled nitric oxide, FeNO), in persistent asthmatics, receiving inhaled corticosteroid therapy.
2. To examine the relationships of mannitol AHR, methacholine AHR and FeNO as continuous variables, to confirm or refute any relationships found as categorical variables.
3. To examine the relationship between mannitol AHR and a further surrogate marker of allergic inflammation, namely salivary eosinophilic cationic protein.

ABSTRACT

Background

Mannitol is a novel osmotic indirect bronchial challenge agent used to aid asthma diagnosis and management, and is thought to reflect underlying inflammatory processes in asthma.

Objective

To evaluate relationships between mannitol AHR and other measures of airway inflammation as well as direct-acting methacholine challenge in persistent asthmatics receiving inhaled corticosteroids.

Methods

We analysed screening data of mild to moderate persistent asthmatics, all receiving ICS, who had mannitol and/or methacholine challenges, FeNO and salivary ECP performed as part of the same screen. Mannitol AHR was grouped by PD_{10}: mild (315-635mg); moderate (75-315mg); severe (0-75mg). FeNO groups: low (<25ppb); medium (25-50ppb); high (>50ppb). Methacholine PC_{20} groups: mild (2-8mg/ml); moderate (0.5-2mg/ml); severe (0-0.5mg/ml).

Results

Mannitol PD_{10} groups were significantly different overall for FeNO ($p=0.023$): 43% higher in the severe vs. mild group. There was a significant overall difference for methacholine PC_{20} ($p=0.006$): a 2.1 doubling dilution difference between severe vs. mild mannitol groups. FeNO groups were significantly different overall for mannitol

PD_{10} (p=0.01) and methacholine PC_{20} (p=0.029). Methacholine PC_{20} groups were significantly different overall for mannitol PD_{10} (p<0.001) and FeNO (p=0.005). No significant differences were found across any groups for: salivary ECP, FEV1%, or ICS dose. Mannitol PD_{10}, methacholine PC_{20} and FeNO as continuous variables all correlated with each other.

Conclusions

Mannitol challenge reflects both underlying inflammation using FeNO and direct AHR using methacholine. Thus mannitol may be a useful screening tool for the assessment of asthmatic patients receiving inhaled corticosteroids.

Ethics reference: NFB/FB/192/03

ClinicalTrials.gov reference: NCT01216579

INTRODUCTION

Mannitol is a novel 'indirect' bronchial challenge agent used as an aid to the diagnosis and management of asthma[132,209,268]. The two main hallmarks of asthma are airway inflammation and airway hyper-responsiveness to a variety of stimuli. Mannitol is an osmotic agent that causes a dehydrating effect on the airway epithelium leading to release of inflammatory mediators from inflammatory cells in the bronchial mucosa, ultimately leading to airway smooth muscle contraction and airway narrowing[269]. Similar indirect challenge agents include adenosine monophosphate (AMP) and hypertonic saline. These are in contrast to 'direct' bronchial challenge agents such as histamine and methacholine[20] used to detect airway hyper-responsiveness, which when inhaled, act directly on airway smooth muscle receptors to cause airway narrowing, i.e. identifying smooth muscle sensitivity to the exogenous agent.

The use of mannitol as a bronchial challenge agent has increased in recent years both because of its ease of administration as well as the relation this challenge bears to exercise induced bronchoconstriction[270] and to a lesser degree clinical asthma[271]. Mannitol does not require extensive equipment, nor is it time consuming to perform the challenge; rather a simple spirometer and a trained staff member who can instruct in the use of the dry powder inhaler that is used to administer the mannitol in standard incremental doses are all that are required. In addition, there is increasing evidence to suggest that achieving control of asthma through monitoring airway inflammation and adjusting treatment based on this[6], rather than just simple spirometry and symptoms, may provide an 'early warning system' or 'inflammometer' before asthma symptoms worsen or exacerbations[14] occur.

Previous studies have demonstrated relationships of mannitol challenge with other bronchial challenge tests and markers of airway inflammation. Unsurprisingly mannitol correlates well with other 'indirect' challenges including AMP, hypertonic saline, exercise and eucapnic hyperpnoea[217,272,273]. Mannitol also correlates well with histamine challenge[274] and methacholine challenge[275]. There have been differing reports on the relationship between mannitol and FeNO dependent on whether or not patients are receiving inhaled corticosteroids (ICS), i.e. the relationship seems more robust when ICS are not used[274,276]. The theory that mannitol more closely reflects airway inflammation is also supported by its strong correlation not only with FeNO but also with sputum eosinophils in steroid naïve patients as compared to methacholine which did not correlate with eosinophils in the same study[131].

Mannitol has been recently shown to have a sensitivity and specificity similar to methacholine challenge for the diagnosis of asthma[275] along with a similar negative predictive value to fractional exhaled nitric oxide (FeNO) levels using a FeNO cut-off of <36ppb to exclude asthma in a steroid naïve group[275].

As a clinician it is clearly useful to be able to quickly and easily quantify the degree of severity of a test and how this corresponds to the underlying condition in question, particularly a test that is becoming more widely used. There are no standardised guidelines on severity grading of mannitol challenge as there are for methacholine[20], and more recently for FeNO[212], from the American Thoracic Society. Therefore, our objective was to further explore the relationships of mannitol AHR with other markers of inflammation in asthma, as well as methacholine challenge, by using cut-off values to help describe AHR to mannitol as mild, moderate or severe in addition to how

these groups relate to previously reported cut-off values for both FeNO[212] and methacholine challenge[277]. We also examined the relationship between these mannitol grades and salivary eosinophilic cationic protein (ECP) as a further surrogate marker of allergic inflammation in persistent asthma.

METHODS

We performed analysis of data from patients who attended the Asthma and Allergy Research Group for screening prior to a clinical trial[6] who had a diagnosis of mild to moderate persistent asthma, all of whom were regularly taking inhaled corticosteroids, and had either or both mannitol and methacholine challenges performed within a 1 week period. Each individual had given their consent for their information to be held in our database and used for research purposes. Tayside Medical Research Ethics Committee granted ethical approval.

Patients either had a mannitol or methacholine challenge performed at screen along with spirometry, exhaled tidal nitric oxide levels, skin prick testing, salivary ECP quantification, and demographic data including: age, gender, smoking status and ICS dose. Patients who had both challenges performed as part of their screening process had the second challenge within 1 week of the initial visit because this could not be performed at the same time as the initial challenge. Patients were required to have a baseline forced expiratory volume in 1s (FEV_1) ≥60% predicted to ensure safety during both bronchial challenge tests as per our departmental policy.

Groups were generated based on AHR to mannitol PD_{10} (cumulative provocative dose required to produce a 10% fall in FEV_1) with cut-off values in line with specific dose increments (mild: 315-635mg; moderate: 75-315mg; severe: 0-75mg); and to methacholine provocative concentration of methacholine required to cause a 20% fall in FEV_1 (PC_{20}) (mild: 2-8mg/ml; moderate: 0.5-2mg/ml; severe: 0-0.5mg/ml) – these are related to a previous study we performed using these cut-off values[277] and are closely related to but not exactly the same as the ATS severity levels[20]. We also

grouped FeNO as per the recent ATS guidelines (low: <25ppb; medium: 25-50ppb; high: >50ppb)[212]. The lower limit of each group comprised exact numbers; the exact number for the upper limit belonged to the next highest group.

We used mannitol PD_{10} rather than PD_{15} (which is the standard measurement in diagnosis for steroid-naïve patients) for several reasons. PD_{10} provided a greater number of responsive patients (i.e. below 635mg) in order that we could more accurately assess correlations between the different measurements. Moreover, these were patients already on inhaled corticosteroids; therefore, we believed there would be a greater signal using PD_{10} over PD_{15} for these patients – indeed in the STAMINA study[6] mannitol PD_{10} was used a priori as the experimental method of ICS dose titration showing benefits on exacerbations and other outcomes. To validate this method, we have performed a correlation between mannitol PD_{10} to PD_{15} (for those patients who reached this) in this cohort.

Study measurements

Mannitol challenge was performed[217] with a dry powder inhaler (Aridol Pharmaxis Ltd, Sydney, Australia) using increments up to a maximum cumulative dose of 635mg. The PD_{10} was calculated by interpolation of the log-linear dose-response curve. Results from patients who did not respond to mannitol by failing to reach a PD_{10} at the highest cumulative dose, i.e. censored at >635mg, were imputed to 1270mg (double the maximum dose) for statistical purposes. A SuperSpiro spirometer (Micro Medical Ltd., Chatham, Kent, UK) was used to perform spirometry in accordance with European Respiratory Society (ERS) guidelines[216]. Methacholine was dispensed via a nebulised solution of methacholine (Tayside Pharmaceuticals,

Dundee, UK), and the challenge was performed in accordance with American Thoracic Society (ATS) guidelines[20]. A Mefar dosimeter was used with doubling concentrations from 0.03 to 32 mg/ml. The PC_{20} was then calculated. FeNO was performed using a NIOX chemiluminescence analyser (Aerocrine AB, Solna, Sweden) at 50 ml/s in accordance with the published guidelines[22]. Skin prick testing with eight common aeroallergens (Merck, Middlesex, UK) was performed. Salivary eosinophilic cationic protein (ECP) was quantified in duplicate using an enzyme linked immunoassay technique (UniCAP; Sweden Diagnostics UK Ltd, Milton Keynes, UK) with an intra-assay co-efficient of variation of 3.3% and inter-assay co-efficient of variation of 4%.

Statistical analysis

All data were checked for normality of distribution prior to analysis. Any non-Gaussian data were logarithmically transformed to achieve normality prior to analysis. Any data that could not achieve a normal distribution with transformation were analysed using the equivalent non-parametric statistical tests. Primary analysis was based on comparisons of the categories for severity of mannitol AHR, as well as methacholine AHR and FeNO groups, against outcome variables using an overall ANOVA for multiple means comparisons (or independent samples Kruskal-Wallis test for non-parametric outcome variables). Confirmatory analyses were performed using Pearson's and Spearman's correlations (parametric and non-parametric variables respectively) for mannitol PD_{10}, methacholine PC_{20}, and FeNO all as continuous variables, with each other, as well as salivary ECP, smoking status, inhaled corticosteroid dose, age, gender, and number of positive skin prick tests. SPSS version 18.0 (SPSS Inc., Chicago, IL) was used for all analyses.

RESULTS

143 patients with a physician diagnosis of persistent asthma were identified for this study from our records. 134 patients had a mannitol challenge of which 123 patients (92%) had a positive response with a mannitol PD_{10}≤635mg. 111 patients had a methacholine challenge performed of which 99 patients (89%) responded positively with a PC_{20}≤8mg/ml. 100 patients had both mannitol and methacholine challenges performed of which 87% were positive to both at the levels described above. Mean age was 53.7years, 51% were female. 79% of patients with skin prick tests were positive. Patients had well preserved lung function with mean FEV_1 85.7% predicted. Mean ICS dose as BDP equivalent was 345µg/day (95% CI: 310, 379). All demographic variables can be seen in Table 15.

Table 15. Baseline demographics.

Variable	N=143
Age (years)	53.7 (51.3, 56.1)
Gender (Female %)	51.0
Smoking Status (yes, no, %)	3.5, 96.5
FEV_1 (L)	2.5 (2.39, 2.61)
FEV_1 (% predicted)	85.7 (83.1, 88.3)
ICS Dose (µg/day)	345 (310, 379)
Mannitol PD_{10}* (mg, n=134)	137.6 (108.2, 175.0)
FeNO* (ppb)	26.2 (22.6, 30.5)
Meth PC_{20}* (mg/ml, n=111)	1.19 (0.8, 1.78)
ECP* (µg/L)	14.4 (11.5, 17.9)

Data presented as arithmetic mean with 95% confidence intervals unless stated. *Geometric mean with 95% confidence intervals. FEV_1=forced expiratory volume in 1 second. ICS=inhaled corticosteroid as beclometasone dipropionate equivalent dose. PD_{10}=provocative dose of mannitol causing 10% fall in FEV_1. FeNO=fractional exhaled nitric oxide. ppb=parts per billion. Meth PC_{20} =methacholine provocative concentration causing 20% fall in FEV_1. ECP=Salivary eosinophilic cationic protein.

Categorical Analysis by Mannitol AHR

There were significant overall differences between mannitol PD_{10} groups for FeNO levels (p=0.023) with FeNO rising across the 3 groups from 'mild' through to 'severe' mannitol AHR (Table 16, Figure 17). FeNO levels were 43.5% (95% CI: 14.9, 62.5) higher in the severe mannitol AHR group than in the mild group. There were also significant differences across mannitol groups for methacholine PC_{20} (p=0.006) with greater AHR to methacholine demonstrated from mild through to severe mannitol AHR (Table 16, Figure 17) – there was a 2.09 (95% CI: 0.58, 3.60) doubling dilution reduction in methacholine PC_{20} between mild and severe mannitol groups. There were no significant differences between mannitol AHR groups for: salivary ECP (Table 16, Figure 17); number of positive skin prick tests; FEV_1 %predicted (Table 16, Figure 17); or ICS dose (Table 16).

Table 16. Mannitol groups.

Mannitol PD_{10} (mg)	**Mild (315-635)**	**Moderate (75-315)**	**Severe (0-75)**	**P-value**
FeNO (ppb)	20.4 (15.5, 27.0)	24.7 (19.0, 32.2)	36.1 (26.7, 48.8)**	0.023†
Meth PC_{20} (mg/ml)	1.98 (1.15, 3.41)	1.20 (0.79,1.83)	0.47 (0.20, 1.06)***	0.006†
ECP (µg/L)	12.5 (7.8, 19.9)	14.8 (10.1, 21.8)	14.1 (9.0, 22.1)	0.842†
FEV_1* (% predicted)	88.7 (83.3, 94.2)	84.1 (79.5, 88.7)	83.9 (78.7, 89.0)	0.350†
ICS dose* (µg/day)	382 (305, 461)	333 (280, 386)	355 (282, 428)	0.495‡

Data presented as geometric mean with 95% confidence intervals in parenthesis unless stated. *Arithmetic mean (95% confidence intervals). FEV_1=forced expiratory volume in 1 second. ICS=inhaled corticosteroid as beclometasone dipropionate equivalent dose. PD_{10}=provocative dose of mannitol causing 10% fall in FEV_1. FeNO=fractional exhaled nitric oxide. ppb=parts per billion. Meth PC_{20} =methacholine provocative concentration causing 20% fall in FEV_1. ECP=Eosinophilic cationic protein. †Means comparison with overall analysis of variance (ANOVA). ‡Comparison of distributions with independent samples Kruskal-Wallis test. **p<0.05 pairwise comparison (after Bonferroni correction) to mild group. ***p<0.01 pairwise comparison (after Bonferroni correction) to mild group. Significance set at p<0.05 for all analyses.

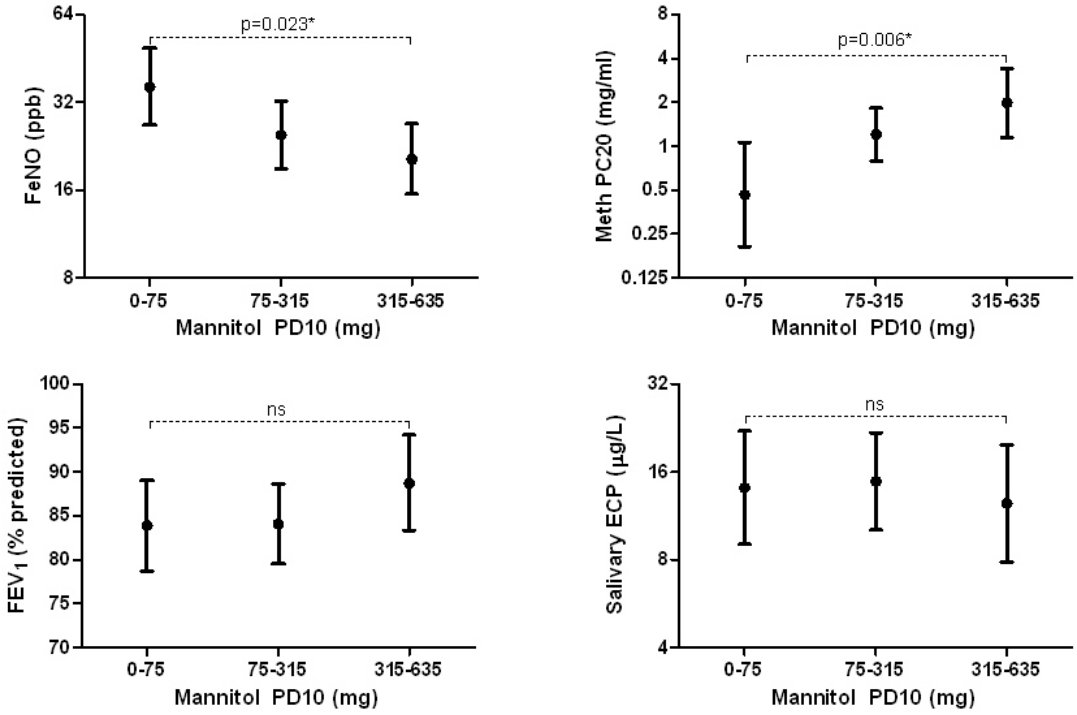

Figure 17. Mannitol PD10 airway hyper-responsiveness groups.

Versus: FeNO (top left); Methacholine PC_{20} (top right); Forced Expiratory Volume in 1 second (FEV_1) as % of predicted (bottom left); and salivary ECP (bottom right). Data presented as geometric means with 95% confidence error bars for FeNO, Meth PC_{20} and ECP. FEV_1 data presented as arithmetic means with 95% confidence error bars. *ANOVA comparisons with significance at $p<0.05$. PD_{10}=provocative dose (of mannitol) causing 10% fall in FEV_1. FeNO=fractional exhaled nitric oxide. Meth PC_{20}=provocative concentration (of methacholine) causing 20% fall in FEV_1. ECP=Eosinophilic cationic protein. ppb=parts per billion.

Categorical Analysis by Exhaled Nitric Oxide

There were significant overall differences between FeNO groups for mannitol PD_{10} (p=0.01) with a reduction in mannitol AHR as FeNO levels reduced (Table 17, Figure 18). There was a 1.14 (95% CI: 0.31, 1.97) doubling dose increase in mannitol PD_{10} between highest FeNO (>50ppb) and lowest FeNO (<25ppb) groups. Also there were significant differences across FeNO groups for methacholine PC_{20} (p=0.029) with a 2.08 (95% CI: 0.60, 3.55) doubling dilution increase in PC_{20} between highest (>50ppb) and lowest (<25ppb) FeNO groups (Table 17, Figure 18). There were no

significant differences between FeNO groups for: salivary ECP (Table 17, Figure 18); number of positive skin prick tests; FEV_1 %predicted (Table 17, Figure 18); or ICS dose (Table 17).

Table 17. Fractional exhaled nitric oxide groups.

FeNO Grade (ppb)	Severe >50	Moderate 25-50	Mild <25	p-value
Mannitol PD_{10} (mg)	80 (49, 131)	199 (122, 324)**	177 (126, 248)**	0.01†
Meth PC_{20} (mg/ml)	0.49 (0.27, 0.89)	1.23 (0.47, 3.25)	2.09 (1.08, 4.03)**	0.029†
ECP (µg/L)	12.3 (7.4, 20.5)	12.7 (7.6, 21.2)	21.0 (15.0, 29.4)	0.106†
FEV_1* (% predicted)	86.5 (80.7, 92.3)	85.0 (78.6, 91.5)	86.3 (82.6, 90.1)	0.922†
ICS dose* (µg/day)	299 (242, 356)	355 (283, 426)	367 (311, 423)	0.392‡

Data presented as geometric means (95% confidence intervals) unless stated. *Arithmetic mean (95% confidence intervals). FEV_1=forced expiratory volume in 1 second. ICS=inhaled corticosteroid as beclometasone dipropionate equivalent dose. PD_{10}=provocative dose of mannitol causing 10% fall in FEV_1. FeNO=fractional exhaled nitric oxide. ppb=parts per billion. Meth PC_{20} =methacholine provocative concentration causing 20% fall in FEV_1. ECP=Eosinophilic cationic protein. †Means comparison with overall analysis of variance (ANOVA). ‡Comparison of distributions with independent samples Kruskal-Wallis test. **$p<0.05$ pairwise comparison (Bonferroni correction) to severe group. Significance set at $p<0.05$ for all analyses.

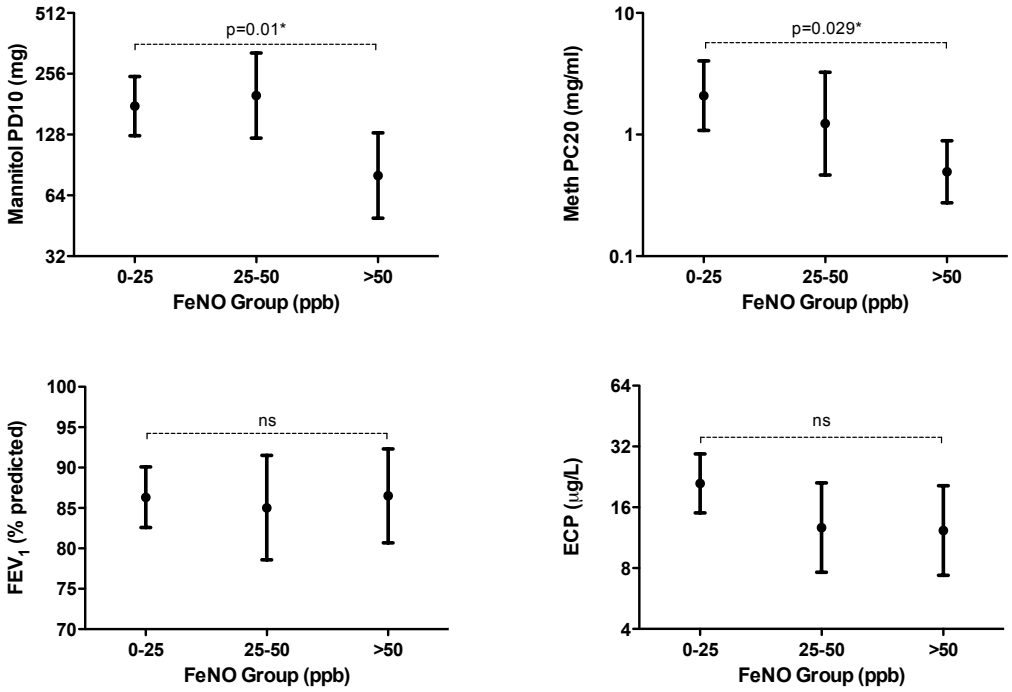

Figure 18. FeNO airway inflammation groups.

Versus: Mannitol PD_{10} (top left); Methacholine PC_{20} (top right); Forced Expiratory Volume in 1 second (FEV_1) as % of predicted (bottom left); and salivary ECP (bottom right). Data presented as geometric means with 95% confidence error bars for Mannitol PD_{10}, Meth PC_{20} and ECP. FEV_1 data presented as arithmetic means with 95% confidence error bars. *ANOVA comparisons with significance at $p<0.05$. PD_{10}=provocative dose (of mannitol) causing 10% fall in FEV_1. FeNO=fractional exhaled nitric oxide. Meth PC_{20}=provocative concentration (of methacholine) causing 20% fall in FEV_1. ECP=Eosinophilic cationic protein. ppb=parts per billion.

Categorical Analysis by Methacholine AHR

There were significant overall differences between methacholine AHR groups for mannitol PD_{10} ($p<0.001$) with mannitol AHR reducing as methacholine AHR reduced (Table 18, Figure 19). There was a 2.07 (95% CI: 1.19, 2.95) doubling dose increase in mannitol PD_{10} between severe (<0.5mg/ml) through to mild (2-8mg/ml) methacholine AHR groups. There was also a significant difference across methacholine AHR groups for FeNO levels ($p=0.005$) with FeNO reducing as

methacholine AHR reduced with a 51.6% (95% CI: 23.4, 69.4) reduction in FeNO levels between severe and mild methacholine AHR groups (Table 18, Figure 19). There were no significant differences between methacholine AHR groups for: salivary ECP (Table 18, Figure 19); number of positive skin prick tests; FEV_1 %predicted (Table 18, Figure 19); or ICS dose (Table 18).

Table 18. Methacholine groups.

Meth PC_{20} Grade (mg/ml)	**Severe <0.5**	**Moderate 0.5-2**	**Mild >2-8**	**P-value**
Mannitol PD_{10} (mg)	57 (35, 92)	147 (89, 242)**	239 (161, 354)***	<0.001†
FeNO (ppb)	38.7 (28.5, 52.4)	25.1 (19.1, 32.9)	18.7 (13.2, 26.6)***	0.005†
ECP (µg/L)	13.0 (8.3, 20.3)	19.3 (11.6, 32.3)	12.1 (7.1, 20.8)	0.354†
FEV_1* (% predicted)	87.1 (82.2, 92.0)	86.4 (81.6, 91.1)	89.5 (83.9, 95.2)	0.677†
ICS dose* (µg/day)	314 (246, 382)	312 (266, 358)	390 (301, 479)	0.152‡

Data presented as geometric means (95% confidence intervals) unless stated. *Arithmetic mean (95% confidence intervals). FEV_1=forced expiratory volume in 1 second. ICS=inhaled corticosteroid as beclometasone dipropionate equivalent dose. PD_{10}=provocative dose of mannitol causing 10% fall in FEV_1. FeNO=fractional exhaled nitric oxide. ppb=parts per billion. Meth PC_{20} =methacholine provocative concentration causing 20% fall in FEV_1. ECP=Eosinophilic cationic protein. †Means comparison with overall analysis of variance (ANOVA). ‡Comparison of distributions with independent samples Kruskal-Wallis test. **p<0.05 pairwise comparison (Bonferroni correction) to severe group. ***p<0.01 pairwise comparison (Bonferroni correction) to severe group. Significance set at p<0.05 for all analyses.

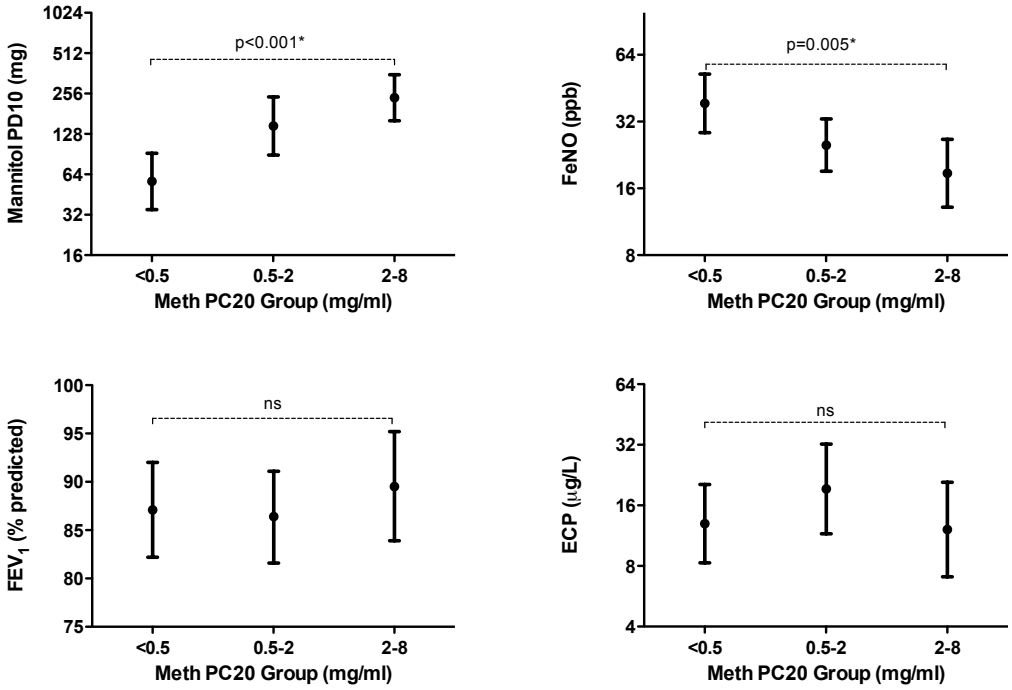

Figure 19. Methacholine PC_{20} airway hyper-responsiveness groups.

Versus: Mannitol PD_{10} (top left); FeNO (top right); Forced Expiratory Volume in 1 second (FEV_1) as % of predicted (bottom left); and salivary ECP (bottom right). Data presented as geometric means with 95% confidence error bars for Mannitol PD_{10}, FeNO and ECP. FEV_1 data presented as arithmetic means with 95% confidence error bars. *ANOVA comparisons with significance at $p<0.05$. PD_{10}=provocative dose (of mannitol) causing 10% fall in FEV_1. FeNO=fractional exhaled nitric oxide. Meth PC_{20}=provocative concentration (of methacholine) causing 20% fall in FEV_1. ECP=Eosinophilic cationic protein. ppb=parts per billion.

Continuous Data Analysis

Modest significant correlations were found between mannitol PD_{10} as a continuous variable versus FeNO (r=-0.209, p=0.025), (Figure 20a) and versus methacholine PC_{20} (r=0.463, p=<0.001), (Figure 20b). There were also significant correlations between methacholine PC_{20} versus FeNO (r=-0.367, p<0.001) and methacholine PC_{20} versus ICS dose (r=0.282, p=0.005). Salivary ECP also correlated with number of

skin prick tests (r=0.389, p=0.003). There were no other significant correlations of mannitol PD_{10}, FeNO or methacholine PC_{20} with any remaining variables.

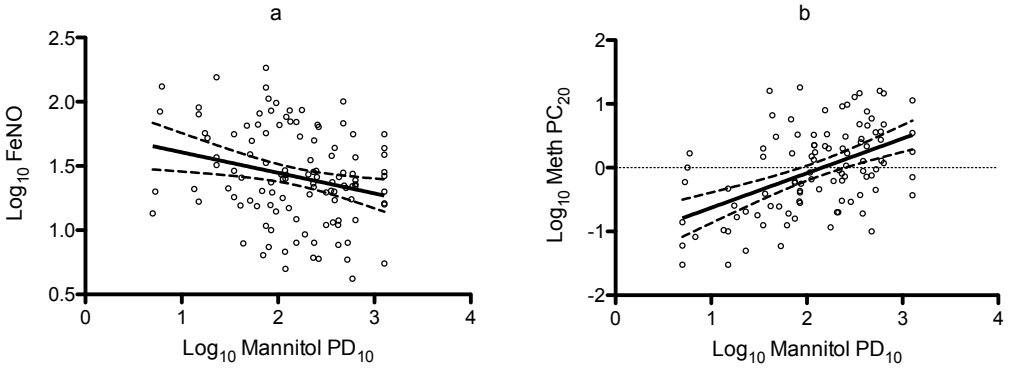

Figure 20. Correlations between (a) Mannitol PD_{10} vs. FeNO and (b) Mannitol PD_{10} vs. Methacholine PC_{20}.

Analysis with Pearson's test following logarithmic transformation of all variables. Solid line represents line of identity with interrupted lines 95% confidence intervals.

There was a very strong correlation between mannitol PD_{10} and mannitol PD_{15} (in those patients who also demonstrated this greater degree of mannitol AHR, i.e. provocative dose of mannitol causing 15 % fall in FEV_1), Figure 21.

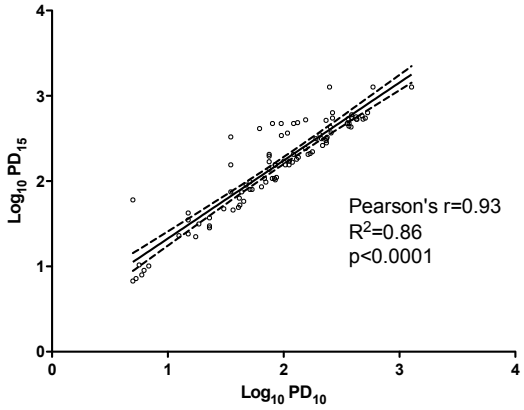

Figure 21. Correlation between mannitol PD_{10} and PD_{15} in subgroup with both values.

Analysis with Pearson's test following logarithmic transformation of both variables. Solid line represents line of identity with interrupted lines 95% confidence interval. r = correlation coefficient. R^2 = goodness of fit of line of identity. Significance set at $p<0.05$.

DISCUSSION

This study has sought to categorise the response to mannitol bronchial challenge in relation to other measures of allergic inflammation, and to direct AHR measurement using standard methacholine challenge in patients taking ICS for persistent asthma.

We have found that mannitol AHR grouped by PD_{10}, showed significant relationships with both FeNO levels and methacholine PC_{20}. The corollary was also true - in that previously described groupings for FeNO[212] and methacholine PC_{20}[277] were both significantly related to mannitol PD_{10} in our cohort. These significant correlations also remained when all three measures (mannitol PD_{10}, FeNO and methacholine PC_{20}) were analysed as continuous variables. This clearly demonstrates that mannitol bronchial challenge is related to both classical hallmarks of clinical asthma, i.e. airway inflammation (FeNO) and AHR (methacholine). Furthermore, the magnitudes of differences between our mild and severe mannitol groups for these outcome variables were clinically relevant - a 2.1 doubling dilution difference for methacholine PC_{20}, and a 43% difference in FeNO. Pointedly such differences were seen despite patients taking concomitant ICS therapy, suggesting that patients may not need to stop their ICS prior to mannitol challenge.

Despite finding a significant relationship between our mannitol AHR groups and FeNO, which is a non-specific measure of whole lung inflammation, there were no such relationships between our mannitol groups with salivary ECP, also used to reflect the allergic inflammatory process in asthma. One explanation for the disconnect between mannitol and salivary ECP could be current inhaled corticosteroid use. It may be that salivary ECP is much more responsive to low doses of ICS than

FeNO, meaning that ECP reaches a much earlier plateau in response than other markers. This theory is supported by the absolute values for salivary ECP in our cohort, which were rather low compared to other studies that used this as a biomarker of asthma[278,279]. It may also be that salivary ECP does not correlate with ECP in sputum from the airways[280].

It has previously been shown that FeNO bears a stronger correlation to mannitol than it does to methacholine[131] in steroid naïve asthmatics. Whilst we have demonstrated a correlation between FeNO and mannitol, the correlation between FeNO and methacholine is stronger in the present study, which may be due to FeNO suppression with ICS. However, despite the expectation that ICS would suppress FeNO even at relatively low doses[221], it seems that FeNO still provides a signal of airway inflammation on ICS, albeit this is attenuated. We have previously reported a relationship between methacholine and FeNO in a population of asthmatics with different demographics[277] than reported here, and reassuringly the results are similar in this regard.

Our study also confirms that measurement of airway calibre (as pulmonary function) in mild to moderate asthmatics is not useful in determining degree of severity of the underlying airway inflammation and hyper-responsiveness, with no correlation found between FEV_1 (% predicted) or any of mannitol AHR, FeNO, or methacholine AHR. The main reason for this is that FEV_1 tends to be well preserved in mild to moderate asthmatics as we have once again demonstrated in our cohort.

Previous studies have shown that mannitol AHR correlates both with FeNO and methacholine[131,275]. Indeed mannitol is almost equivalent in terms of its sensitivity and specificity for diagnosing asthma as methacholine[275]. Given the relationships we have also found, one wonders if mannitol should be the initial screening investigation of choice, particularly since current standard screening employs spirometry, yet mannitol challenge is a simple and relatively inexpensive challenge test that clearly bears more relation to the hallmarks of asthma than spirometry for mild to moderate patients. Previously, mannitol PD_{15} has been the standard used as the diagnostic cut-off for this challenge. However, not only have we demonstrated a strong significant correlation between mannitol PD_{10} and PD_{15} (in the subgroup of patients who reached this), it is possible that 'stratifying' asthmatic patients already on ICS may be more accurately performed with PD_{10}.

The strengths of this study are the prospectively collected, 'real life', screening data of mild to moderate asthmatics on doses of ICS that accurately reflect community prescribing practices. The size of our cohort was moderately large, presenting us with a good cross-section of asthmatic patients. There were very few current smokers (3.4%) making confounding of our findings from this source unlikely. A limitation of this study could be our reliance on patient reporting about compliance to their ICS therapy prior to screening. In addition to this we had no joint mannitol/methacholine screening data on patients who were not taking ICS, which would have been helpful given that mannitol challenge could be considered a *de novo* diagnostic test. However, what we have shown confirms that mannitol challenge is important when it comes to monitoring disease control given its relation to the hallmarks of asthma, once it has been diagnosed. Finally, other easier to measure biomarkers of underlying

inflammation may be equally or more useful in stratifying asthma. For example serum high sensitivity C-reactive protein has been shown to correlate well with both FeNO and serum ECP in steroid naïve asthmatics, and it is also present in much higher levels in unstable asthma[281]. However, this test was not part of our battery of screening tests at the time of collecting this data.

In conclusion, we have identified categories of mannitol AHR which are correlated to airway inflammation as measured by FeNO as well as to direct AHR using methacholine challenge in mild to moderate persistent asthmatics currently taking inhaled corticosteroids. This might provide a useful guide to gauge the degree of both airway inflammation and airway hyper-responsiveness in persistent asthmatics. We also found that mannitol AHR is not associated with salivary ECP or to airway calibre in mild to moderate asthmatics. These findings help to add weight to the argument that mannitol challenge is related to local airway allergic inflammation and hyper-responsiveness in persistent asthma. However, further prospective studies using these categories for mannitol AHR (as PD_{10}) compared to other outcome measures of asthma are warranted, such as exacerbations or asthma control, including those patients on higher doses of inhaled corticosteroids.

CHAPTER 7: INHALED CORTICOSTEROID DOSE RESPONSE IN ASTHMA: SHOULD WE MEASURE INFLAMMATION?

Study aims:

1. To compare dose response relationships of ICS between measures of pulmonary function, symptoms and inflammation in persistent asthmatics.
2. To compare these ICS dose response relationships using common dose ramps of ICS.
3. To identify where ICS dose titration provides the greatest impact for a given individual's treatment in order to achieve optimal asthma control.

ABSTRACT

Background

Inhaled corticosteroid (ICS) titration in asthma is primarily based on symptoms and pulmonary function. ICS may not be increased on this basis despite residual airway inflammation.

Objective

To compare the dose-response relationships of ICS on measures of pulmonary function, symptoms and inflammation in persistent asthmatics.

Methods

We performed a pooled post-hoc analysis of 121 patients with mild-moderate asthma, from four randomised controlled trials that incorporated an ICS dose ramp. Dose ramps were: 0-200µg/day, 0-800µg/day, 200-800µg/day (beclomethasone equivalents). Outcome measures included: spirometry, fractional exhaled nitric oxide (FeNO), airway hyper-responsiveness (AHR), symptoms, serum eosinophilic cationic protein (ECP), and blood eosinophils (Eos).

Results

We found a plateau beyond a small improvement at 0-200µg for forced expiratory volume in 1s (FEV_1, %predicted): 0-200µg, 3.3% (95%CI: 2.0,4.7) vs. 200-800µg, 0.3% (95%CI: -0.8,1.4), P=0.001. A similar plateau was seen for symptom improvement beyond 0-200µg. Inflammatory and AHR outcomes showed further room for improvement beyond low dose ICS. There was dose related suppression

(P<0.0001) for FeNO: ICS free, 40.4ppb (95%CI: 34.7,46.9); 200µg, 26.8ppb (95%CI 23.4,30.2); 800µg, 20.8ppb (95%CI: 18.8,23.1). ECP was significantly reduced with both higher dose ramps. Eos also improved across all three dose ramps, with dose separation between ICS free, 370cells/µL (95%CI: 280,450), vs. 800µg, 250cells/µL (95%CI: 200,300), P=0.03. AHR improved with all three dose ramps, with greater improvement at lower doses for indirect versus direct challenges.

Conclusion

ICS dose-response extends beyond low dose for inflammation and AHR, but not symptoms or spirometry. Further study is required to identify whether this correlates with sub-optimal longitudinal asthma control.

Ethics references: NFB/FB/192/0311, 09/S0501/5223, 11/ES/003125, 08/S1402/1424

ClinicalTrials.gov references: NCT00667992, NCT00995657, NCT01216579, NCT01544634.

INTRODUCTION

Asthma is a heterogeneous chronic inflammatory disease of global importance[16], which places a significant burden both on individual patients and on health care services, where many patients remain inadequately treated[30,31] with an ongoing attendant mortality[25]. The concept of achieving 'total asthma control'[10] is important for reducing the future risk of exacerbations[28,29,46]. It is therefore imperative that we have robust procedures for accurate diagnosis, measurement of severity, prediction of future risk, along with appropriate personalised treatments to achieve this goal. Nevertheless, present guidelines for the identification and treatment of asthma merely include symptoms and lung function measurements[10,11]. The Royal College of Physicians' recent National Review of Asthma Deaths report[25] found that only 39% of patients who died were actually labelled as having 'severe' asthma according to current guidelines, with the remainder therefore labelled as 'mild' or 'moderate', suggesting we may not be accurately identifying those at greatest risk.

Measurement of inflammatory outcomes has significantly improved the management of asthma, evolving personalised treatment. Studies have consistently shown that titrating steroid therapy against inflammation improves outcomes such as exacerbation rates[6,14,15]. Green et al demonstrated this by titrating steroid treatment against sputum eosinophil counts, resulting in significantly fewer severe exacerbations compared to standard guideline driven treatment[14].

Price et al demonstrated retrospectively, in a primary care cohort, that those with higher blood eosinophil counts fared worse in terms of experiencing more severe exacerbations and poorer asthma control[77]. Moreover, eosinophilic inflammation may

be masked when using a long-acting beta-2 agonist (LABA) as a steroid-sparing agent[282,283]. Sputum and blood eosinophilia in asthma have both been separately shown to predict loss of asthma control and increased exacerbation rates[46,76,151]. This is also true of fractional exhaled nitric oxide (FeNO) levels[155] and airway hyper-responsiveness (AHR)[46], the latter being largely driven by airway inflammation[131]. It is therefore logical that one might wish to control inflammation over and above simply controlling symptoms and lung function – much like controlling asymptomatic hypertension to prevent subsequent cardiovascular sequelae. This is relevant given lung function and lack of symptoms may be deemed normal despite the possibility of an ongoing underlying inflammatory process[39].

We performed a post-hoc pooled analysis of data from four previously published randomised controlled trials (RCTs) where inhaled corticosteroid (ICS) dose titration was used in a prospective manner. Outcome measurements included symptoms, lung function, inflammation and AHR. We then analysed the dose-response relationship to ICS for these outcomes to identify where incremental ICS dosing provides the greatest impact, and thus likely to be most informative when titrating a given individual's treatment to achieve optimal asthma control.

METHODS

Patients

Male and female mild-moderate persistent asthmatics aged 18-65 years receiving ≤1000μg/day ICS, i.e. expressed as a reference dose of large particle beclomethasone dipropionate (BDP) equivalent dose, were recruited to each of four RCTs[1,2,5,6]. For example, large particle HFA-fluticasone 200ug or small particle HFA-beclomethasone 200ug would be equivalent to large particle HFA-BDP 400ug. Their post-run-in baseline measurements are presented in Table 19. Further detailed inclusion and exclusion criteria can be found in each of the reported trials.

Table 19. Baselines post run-in.

Study Baselines	All n=121	FP (ICS Free) n=21	Bud (ICS free) n=72	HFA-BDP (200µg BDP) n=16	CIC (200µg BDP) n=12
Age (yr)	39.8 (37.2,42.4)	36.8	39.6	37.8	48.7
Sex (M:F)	44:77	6:15	29:43	6:10	3:9
FEV_1 (% pred)	85.1 (82.9,87.3)	88.5	82	90.3	89
FEF_{25-75} (% Pred)	65.7 (61.5,69.8)	53.5	70.4	60.3	-
FEV_1/FVC ratio (%)	75.2 (73.7,76.7)	71.2	75.6	74.6	80.5
FeNO (ppb)*	37.3 (32.3,42.6)	72.4	34.7	29.1	25.4 (n=10)
AHR*	-	102mg (57,183) Mann PD_{15}	0.72 mg/ml (0.58,0.90) Meth PC_{20}	1.31mg/ml (0.64,2.69) Hist PC_{20}	59mg (15,233) Mann PD_{10}
ECP (µg/L)* (n=47)	20.5 (15.9,26.3)	18.6	-	22.4	21.9
Eos (cells/µL) (n=37)	330 (280,390)	370	-	290	-
Symptom Score	-	5.8 (5.4,6.2)	0.96¶ (0.79,1.12)	6.2 (5.9,6.5)	6.1 (5.6,6.5)
Screening ICS (BDP, µg/day)	420 (361,479)	440	414	406	436

Baselines post run-in at the given beclomethasone dipropionate (BDP) equivalent doses for: large-particle hydrofluoroalkane (HFA)-fluticasone propionate (FP), large-particle HFA-budesonide (Bud), small-particle HFA-beclomethasone (HFA-BDP), and small-particle HFA-ciclesonide (CIC). Overall means (95%CI), leftmost column. Arithmetic means (95%CI), unless stated. *Geometric mean (95%CI). Symptom scores are the symptom component of the mini-AQLQ, except Bud. ¶Total Symptom Score: 0-no symptoms; 1-mild; 2-moderate; and 3-severe.

Study Design

We performed a post-hoc analysis using data from 4 RCTs[1,2,5,6], each comprising a component where ICS dose ramp effects were examined on a variety of outcomes including: spirometry, FeNO, AHR including both indirect (mannitol) and direct (methacholine, histamine) challenges, asthma symptoms, serum eosinophilic cationic protein (ECP), and blood eosinophil count (Eos). Briefly, one study used large particle hydrofluoroalkane (HFA)-fluticasone propionate (FP) using FeNO as the primary outcome[1]: with an ICS-free run-in, followed by either 200µg or 800ug BDP

equivalent. A second study titrated small particle HFA-ciclesonide against mannitol AHR versus titration using standard British Thoracic Society (BTS) guidelines[6]: patients were selected from both arms of this study where there was an appropriate dose ramp (i.e. BDP equivalent 200-800µg/day). A third study used methacholine AHR as the primary outcome comparing large particle (HFA) and chlorofluorocarbon (CFC) formulations of budesonide[2]: within the HFA arm there was an ICS free washout followed by 200µg/day or 800ug/day BDP equivalent. The fourth study examined whether propranolol was useful as an ICS sparing agent[5], with histamine AHR the primary outcome: the control arm was effectively a dose ramp between small particle HFA-beclomethasone at BDP equivalent doses of 200µg/day or 800ug/day.

Measurements

Extended detail of measurements can be found in each parent study. Briefly, spirometry was measured using a SuperSpiro spirometer (Micro Medical Ltd.) according to American Thoracic Society (ATS) guidelines[123]. Exhaled tidal nitric oxide was measured according to ATS recommendations[22] using a NIOX analyser (NIOX® Nitric Oxide Monitoring System, Aerocrine AB) prior to other pulmonary function measures. Mannitol challenge was performed as previously described[217] with a dry powder inhaler (Aridol; Pharmaxis Ltd) using cumulative dose increments up to 635 mg. Histamine for bronchial challenge was dispensed via nebulized solution with doubling concentrations of histamine from 0.3125mg/ml to 40mg/ml. Methacholine challenge was performed using the five-breath dosimeter technique in accordance within ATS recommendations[20]. Peripheral blood eosinophils were measured using the Sysmex XE2100 Hematology auto-analyser. Serum ECP was

measured in duplicate using a UniCAP system (Phadia) with a coefficient of variation of 3%. For symptoms, 3 studies[1,5,6] included the mini-Asthma Quality of Life Questionnaire (AQLQ, symptom component), where a mean score of 7 indicates no symptoms, and <7 indicates progressively worse symptoms. In the fourth study[2], the following rating scales were used: 0, no asthma symptoms; 1, mild symptoms (easily tolerable); 2, moderate symptoms (interferes with normal activities/sleep); and 3, severe (prevents normal activities/ sleep). The total asthma symptom score was a mean of both morning and evening symptom scores.

Statistical Analysis

All data were initially assessed for normality of distribution, with non-Gaussian distributions logarithmically transformed to enable parametric analyses. For the primary analyses, examining for any change within a given outcome measure following an ICS dose ramp, arithmetic means of the difference within each ICS dose ramp were calculated for outcomes with the same parametric measure across all 4 studies. Geometric mean fold differences were calculated for changes in outcomes that were either non-parametric, or with different measurements between the studies in order to standardize any changes (e.g. bronchial challenges). Analyses of variance (ANOVA) with Bonferroni correction were also used to compare the differences between dose ramp responses, and each individual dose mean. Statistical significance for all comparisons was set at $P<0.05$. IBM SPSS version 22 was used for the statistical analyses.

RESULTS

We included 121 evaluable participants from the parent studies (Table 19). Ciclesonide patients were approximately 10 years older. Patients had generally preserved pulmonary function, overall mean FEV_1 85.1% predicted, and had mild symptoms. FeNO was higher in the Fluticasone group due to the inclusion criteria of that study. Despite the different bronchial challenges, their figures all indicated a moderate-severe degree of AHR. Patients had been receiving similar ICS doses prior to study inclusion, mean 420µg/day BDP equivalent.

For pulmonary function, there were small but statistically significant changes seen within the 0-200µg dose ramp for both FEV_1 and FEF_{25-75} (Table 20, Figure 22), with a 3.3% (95%CI 2.0,4.7) rise in FEV_1 (P<0.0001), and 4.6% (95%CI 2.4,6.9) rise in FEF_{25-75} (P<0.0001). However, there was a plateau in response at 200-800µg for both these measures. There were also statistically significant within-group improvements for symptom scores (Figure 22) at all dose ramps, but with less improvement within the 200-800ug dose ramp: along with a significant difference (P=0.01) when comparing responses between 0-800µg vs. 200-800µg, i.e. again indicating a plateau in response.

Table 20. Pulmonary function and inflammatory outcomes.

ICS BDP (µg/day)	ICS Free (a)	200ug (b)	800ug (c)	P-value (ANOVA)
Pulmonary Function				
FEV_1 (% pred)	83.4 (81.0,85.9)	87.6[§] (85.4,89.9)	87.9[§] (86.0,89.9)	0.01
FEF_{25-75} (% pred)	66.6 (62.0,71.2)	69.6 (65.7,73.5)	70.0 (66.1,73.8)	0.47
Inflammatory Outcomes				
FeNO (ppb)*	40.4 (34.7,46.9)	26.8[#] (23.4,30.2)	20.8[#¶] (18.8,23.1)	<0.0001
ECP (µg/L)*	18.7 (12.1,28.8)	20.2 (15.3,26.5)	13.2 (9.9,17.4)	0.08
Eos (cells/µL)	370 (280,450)	300 (240,350)	250[§] (200,300)	0.04

Data presented as arithmetic means (95% confidence intervals) unless otherwise stated. *Geometric mean (95% CI). [§]P<0.05 vs. (a). [¶] P=0.01 vs. (b). [#]P<0.001 vs. (a).

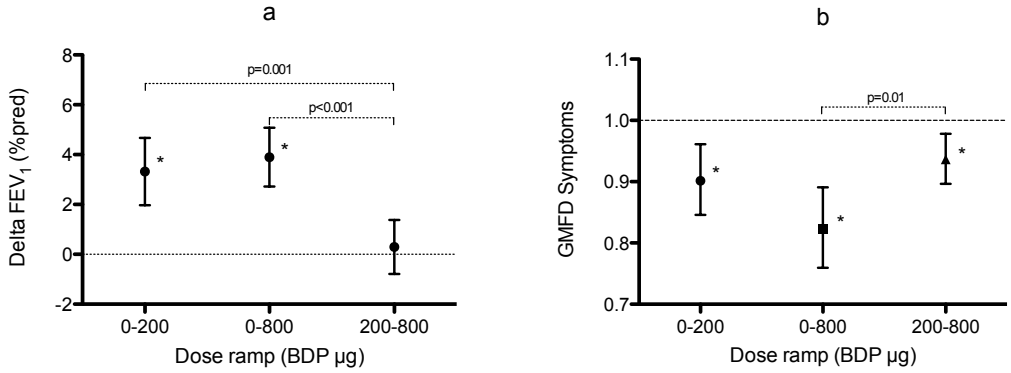

Figure 22. Dose responses for FEV_1 and symptom scores.

(a) mean percentage changes (95%CI error bars) for FEV_1 as %predicted. (b) change in symptom scores as geometric mean fold differences (GMFDs, 95% CI error bars); scores <1 indicate improvement. Asterisk denotes significant within-group change, P<0.05. Remaining P-values compare responses between groups. Where there is no p-value for comparison indicates non-significance.

For inflammation, significant improvements in FeNO were seen across all ICS dose ramps (Table 20, Figure 23a), with clear evidence of dose separation (Table 20). Improvements were more pronounced in the subgroup with baseline values of FeNO≥25ppb (Figure 23b), and significantly different compared to the subgroup with baseline values of FeNO<25ppb. Serum ECP did not improve with the low dose ramp 0-200µg (Table 20, Figure 23c), rather requiring the higher ICS dose to achieve significant within-group improvements between 0-800µg (P=0.002) and 200-800µg (P=0.0002); again the dose ramp responses were significantly different between 0-200µg and 200-800µg (P<0.05). Finally there were significant within group improvements for blood Eos across all dose ramps (Table 20, Figure 23d), where Eos also continued to fall significantly as the ICS dose increased: 370cells/µL (95%CI 280,450) at 0µg, to 250cells/µL (95%CI 200,300) at 800µg, P=0.03.

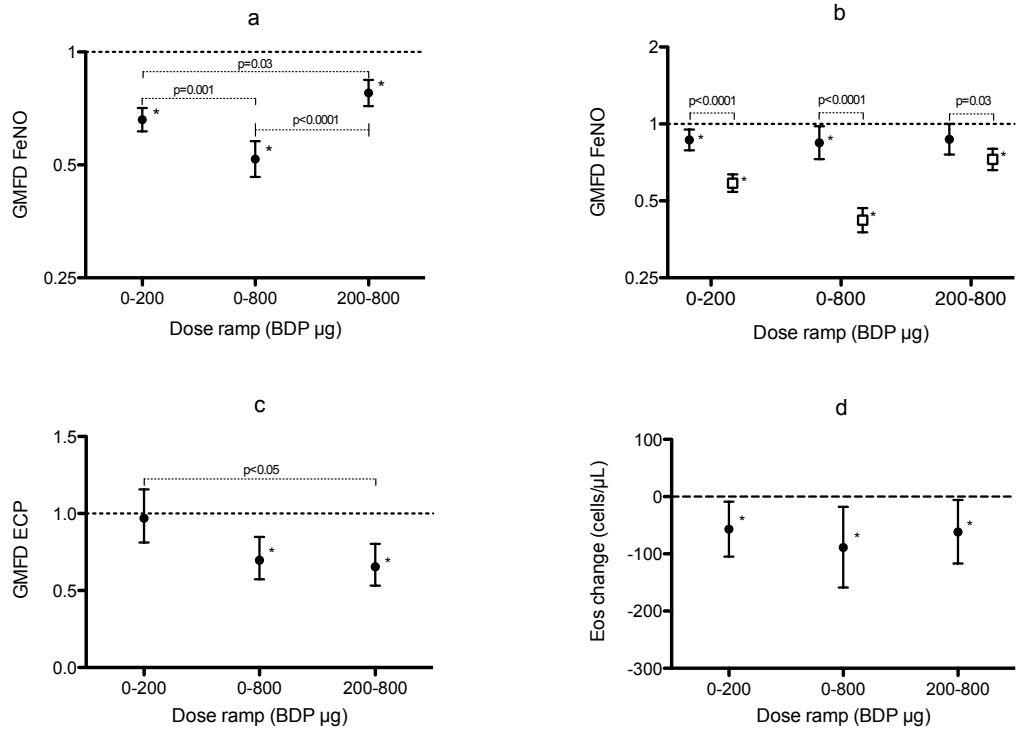

Figure 23. Dose responses for inflammatory outcomes.

Within-group changes (GMFDs, 95%CI) unless stated; scores <1 represent a reduction. (a) FeNO. (b) FeNO comparing baseline FeNO≥25ppb (squares) vs. FeNO<25ppb (circles). (c) Serum ECP. (d) Blood eosinophils, arithmetic mean (95%CI). Asterisk denotes significant within-group difference, P<0.05. Remaining P-values indicate significant between group differences; or significant differences between baseline FeNO groups within each ramp (b), P<0.05.

Significant within group changes were seen across all dose ramps for AHR (Figure 24a). The greatest improvement was in the 0-800µg group at 1.35 doubling dilutions (DD) (95%CI 1.06,1.63), P<0.0001, with further significant improvement in the 200-800µg group amounting to 0.7DD (95%CI 0.43,0.96), P<0.0001. Significantly greater improvements were seen when AHR was separated into indirect (mannitol) versus direct challenges (histamine, methacholine), particularly at the lower dose ramp 0-200µg, 1.64DD (95%CI 0.94,2.35) indirect vs. 0.65DD (95%CI 0.40,0.89) direct, P=0.015 (Figure 24b).

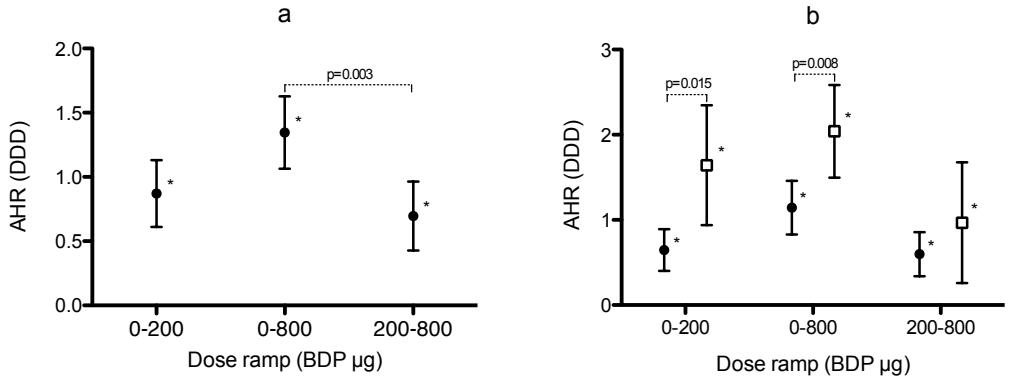

Figure 24. Dose responses in airway hyper-responsiveness (AHR).

Within-group changes expressed as doubling dilution differences (DDD). Scores >0 indicate improvement. (a) combined bronchial challenges. (b) direct (circles) vs. indirect (squares) challenges. Asterisk denotes significant within group change, $P<0.05$. Remaining P-values indicate significant between group differences (a), or significant differences between challenge groups within each ramp (b), $P<0.05$.

DISCUSSION

In the present study we have demonstrated that incremental ICS dosing in persistent asthma leads to small improvements in both pulmonary function and symptoms, which then reaches a plateau above low doses. We have also found that the same ICS dose ramps reveal further room for improvement in both inflammatory outcomes and AHR, when using higher ICS doses up to 800µg/day (beclomethasone equivalent).

The BTS guidelines describe the goal of total (or optimal) asthma control as comprising no symptoms day or night, normal lung function and no exacerbations[10]. Unfortunately, it has been shown that patients are not good at recognizing when their asthma is not controlled[30]. Furthermore, lingering underlying inflammation may be present[39], and until this is treated, symptoms may take many months to become completely normal[40]. We have shown here that patients who have only mild pulmonary function and symptom impairment respond optimally to low doses of ICS for these outcomes. Pointedly, however, the 3.3% rise in FEV_1 for 0-200µg equated to a mean of 98ml (95%CI 57,140), which is less than the putative minimal clinically important difference (MCID) of 230ml in asthma[126]. Moreover, for symptoms, the geometric mean fold difference of 0.94 (95%CI 0.90,0.98) in the 200-800µg group equates to around a 6% improvement. For the mini-AQLQ, for example, 6% represents a change of around 0.4, where the MCID is 0.5[166]. Although the low dose plateau of symptoms and lung function have been well documented[41,44,127,225], we still largely base our asthma management on these two outcomes, in addition to reliever use, particularly for mild to moderate persistent asthma; thus potentially limiting further appropriate ICS escalation.

We have demonstrated room for further improvement in markers of inflammation (FeNO, serum ECP and blood Eos) and AHR with higher doses of ICS, despite the plateau in symptoms and lung function. The presence of ongoing airway inflammation in asthma has been shown to predict not only future exacerbations[152,155], but also loss of asthma control[151] upon reduction or removal of ICS treatment. Furthermore, this has been demonstrated using a variety of inflammatory outcomes including sputum Eos[151,152], blood Eos[77], FeNO[155] and AHR[6,13]. Moreover, targeted ICS therapy towards inflammatory outcomes has been shown to reduce the rate of exacerbations[14], or reduce the overall steroid dose required for total control[15]. We have found that while most improvement in FeNO occurs with low dose ICS, there is still a further dose response to higher ICS doses, particularly in patients who exhibit a high baseline FeNO≥25ppb. This dose response has been shown previously, and indeed when ICS is stopped there is a rebound rise in FeNO once again[159], which alludes to a possible need for persistent over intermittent ICS therapy, or indeed non-adherence to ICS therapy[158].

We saw a similar pattern of response to that of inflammatory outcomes with AHR to a variety of bronchial challenges, both direct and indirect. Indirect challenges are more closely related to the inflammatory pathway as they invoke the inflammatory response in the airway to cause bronchoconstriction[131]. This is perhaps the reason we found a greater magnitude of response for AHR to indirect than direct challenges, in keeping with previous studies[284,285]. Targeting ICS therapy using mannitol challenge AHR has been shown to reduce exacerbations over and above standard guideline driven therapy[6], in line with that for sputum eosinophils[14]. However, AHR can be driven by other mechanisms such as airway smooth muscle hyperplasia, and airway closure

itself[286], and is therefore an area requiring further study with regards to personalised asthma treatment[135].

There is therefore a growing body of evidence suggesting that we need to include inflammatory measurements routinely to best manage patients with asthma. This is problematic, not least by the cumbersome nature of inflammatory and challenge measurements that are difficult to perform in a community setting, albeit measuring blood eosinophils might be part of the solution[77]. Cowan et al. have suggested a panel of inflammatory biomarkers to better enable prediction of ICS responsiveness in asthma[162], but delivery of such a test remains the most difficult hurdle. Even simply using the asthma control questionnaire[33] itself seems to be a good predictor of future risk[29].

We believe the strengths of this study are in the cross-section of patients with mild to moderate disease that we commonly see in the clinic; who additionally use a variety of ICS. Furthermore we believe it has been helpful to study a wide variety of outcome measures, none of which have taken priority in the study design. The limitations of this study are in its post-hoc nature, with relatively low numbers and our ability to only examine low to moderate, albeit commonly used, doses of small and large particle HFA-ICS.

In conclusion, we have demonstrated further room for improvement in markers of inflammation and AHR, despite a plateau in the dose response to ICS for both asthma symptoms and pulmonary function, with small and large particle ICS HFA-formulations and doses. This points to an unmet need of uncontrolled underlying

inflammation in certain asthmatic patients, which may be a precursor to future loss of control.

CHAPTER 8:

PROSPECTIVE FOLLOW-UP OF NOVEL MARKERS OF BONE TURNOVER IN PERSISTENT ASTHMATICS EXPOSED TO LOW AND HIGH DOSES OF INHALED CICLESONIDE OVER 12 MONTHS

Study aims:

1. To identify any systemic adverse effects on sensitive markers of bone turnover comparing low and high doses of inhaled ciclesonide over 12 months in persistent asthmatics.
2. To examine for any effects on adrenal suppression between these ciclesonide doses over 12 months.
3. To confirm the safety of using a strategy of ICS dose titration based on inflammatory airway hyper-responsiveness rather than standard guideline-driven ICS dose titration, where this results in overall higher ICS dosing.

ABSTRACT

Background

In asthmatic patients receiving long-term inhaled corticosteroid therapy, there are concerns regarding the potential for developing systemic adverse effects on bone metabolism, possibly even in the absence of adrenal suppression.

Objective

To investigate whether exposure to inhaled ciclesonide at high verses low doses over one year causes any significant systemic adverse effect on sensitive biomarkers of bone turnover in asthmatic patients.

Methods

We performed a post hoc analysis of stored samples in a subgroup of patients from a prospective, randomised parallel group primary care trial with one year follow-up. 164 mild-moderate persistent asthmatics aged 18-65years with evidence of airway hyper-responsiveness using mannitol bronchial challenge were enrolled into the original study. Of the 119 completed patients per protocol, 100 participants had bone marker samples available for analysis. Ciclesonide was titrated to control persistent asthma against either mannitol bronchial challenge (AHR strategy) or a control group (based on symptoms, reliever use and pulmonary function) over one year. Markers of bone formation (PINP, PIIINP), resorption (ICTP, CTx) and adrenal suppression (overnight urinary cortisol/creatinine: OUCC) at 0 and 12 months were measured.

Results

Mean ciclesonide doses after 12 months were: AHR=507µg/day (n=50); controls=202 µg/day (n=50), $p<0.00001$. There were no significant differences between AHR and control groups at either baseline or after 12 months in PINP, PIIINP, ICTP or CTx; ratios of bone turnover as PINP/ ICTP; PIIINP/CTx; or OUCC.

Conclusion

Higher doses of inhaled ciclesonide do not adversely affect sensitive markers of bone turnover in persistent asthmatics over 12 months.

Ethics reference: NFB/FB/192/03

ClinicalTrials.gov reference: NCT01216579

INTRODUCTION

Inhaled corticosteroids (ICS) are the mainstay of anti-inflammatory therapy in persistent asthma, even in mild disease. During acute exacerbations of asthma, short courses of oral corticosteroid are given for up to two weeks, and in more severe patients long-term use of oral corticosteroids may be required. The use of oral corticosteroids have previously led to a rational concern about what may happen to asthmatic patients with regard to systemic adverse effects such as suppression of their hypothalamic-pituitary-adrenal (HPA) axis, as well as causing detrimental effects on bone metabolism in terms of the potential to develop osteoporosis with increased fracture risk. Indeed, the question of inhaled corticosteroid side effects seems to be increasingly enquired about by well-informed patients during asthma clinic consultations.

It is widely known that systemic corticosteroids, particularly given orally, cause suppression of markers of bone formation[117,118,287], thus altering the balance between bone formation and resorption, leading to an overall reduction in bone mineral density and increased fracture risk. Indeed glucocorticoid induced osteoporosis is the most common cause of secondary osteoporosis[288]. However, the influence of inhaled corticosteroids on bone metabolism is more complex. The degree of systemic bioactivity varies depending on the individual; the dose administered; the pharmacokinetics; and the amount of drug reaching the lung. In general ICS do not cause much problem systemically given at low to medium doses. However, higher doses of certain types of ICS, particularly those that are lipophilic with a large volume of distribution such as fluticasone propionate, can lead to significant HPA axis suppression[49]. For example in a dose ranging study in persistent asthmatics, 440ug

twice daily of inhaled fluticasone was bioequivalent to 8.5mg of oral prednisolone in terms of their propensity for causing adrenal suppression. Current guidelines on corticosteroid-induced osteoporosis delineate a value of 7.5mg prednisolone daily, above which the risk of osteoporotic fracture increases, and bone protection should be considered[289]. Severe asthmatics demonstrate significant peripheral small airways obstruction, which can serendipitously be protective against the systemic effects of high doses of ICS by reducing bioavailability from the distal lung, thus reducing systemic absorption as compared to mild to moderate asthmatics[290]. It has also been demonstrated that high doses of beclometasone dipropionate both increases markers of bone resorption and reduces bone formation as compared to equivalent doses of budesonide[119].

Ciclesonide is a relatively new and novel ICS with several significant pharmacological properties. It has been dubbed a 'designer' third generation ICS[291] because it is a pro-drug with low glucocorticoid receptor activity that is subsequently converted to an active form locally in the lungs that exhibits high glucocorticoid receptor activity[292]. This firstly, has benefits in terms of the pro-drug producing less oropharyngeal candidiasis or laryngeal dysphonia. The active form, des-isobutyryl-ciclesonide (des-CIC) demonstrates significant absorption from the lungs where it is delivered with a high fine particle dose, being an extra-fine aerosol. However, because des-CIC has a very high plasma protein-binding affinity at around 99%, the systemic availability of free active compound is minimal. This has been shown to be effective in producing minimal HPA axis suppression as compared to equivalent therapeutic doses of fluticasone[293-295] creating an enticingly high therapeutic index for this particular drug. The unanswered question, however, is whether there remains a

deleterious effect on bone turnover, in spite of minimal HPA axis suppression with ciclesonide?

In this study we have analysed sensitive serum markers of bone turnover (formation and resorption) as a novel method of investigating any putative systemic adverse effects of inhaled ciclesonide, comparing low and high doses given over one year to patients with mild to moderate persistent asthma. These samples were prospectively collected during a recently published study[6] (the STAMINA trial, ClinicalTrials.gov Identifier: NCT01216579), stored and subsequently analysed after publication once funding became available. To summarise, the STAMINA study compared two separate strategies for titrating ICS dose (as ciclesonide) to achieve asthma control in parallel groups of patients. One was a control group using a standard strategy based on adjusting ICS dose against symptoms, pulmonary function and reliever use, according to conventional British Thoracic Society Guidelines. The other group had their ICS dose adjusted against airway hyper-responsiveness (AHR) using mannitol bronchial challenge as a surrogate for airway inflammation. It demonstrated that the AHR group were exposed to a significantly higher dose of inhaled ciclesonide to achieve superior asthma stability as compared to the control group over the course of one year. Hence this created the ideal environment in which to investigate possible differences in systemic exposure between high and low dose ciclesonide on long-term bone turnover and HPA axis. Nevertheless, ciclesonide was originally chosen for this community-based study because of its high therapeutic index, and lower likelihood of adverse effects (both local and systemic) at higher doses.

METHODS

The methods employed in the reference study have been described previously[6] but we have reiterated the key details and measurements specific to this study with the addition of methods of bone marker analysis.

Patients

164 mild to moderate persistent asthmatics aged 18-65years were initially enrolled from primary care practices within Tayside. Forced Expiratory Volume in 1 second (FEV_1) was required to be >60% of predicted normal to ensure the safety of bronchial challenge testing in a community setting. Patients had to demonstrate AHR following mannitol challenge with a provocative dose causing a 10% drop in FEV_1 (PD_{10}) ≤635mg at the end of a step down period where patients' usual ICS was weaned to the lowest stabilising dose then converted to an equivalent dose of ciclesonide (Alvesco, Nycomed. Konstanz, Germany); the ICS to be used throughout the remainder of the study. All patients had to have been receiving ICS for at least 1 year prior to the study and patients were excluded if they had used oral corticosteroids within 3 months of study commencement, had a prior diagnosis of osteopenia/osteoporosis or were receiving any anti-resorptive therapy. All patients gave written informed consent prior to the study. The study was reviewed and approved by the Tayside Committee on Medical Research Ethics.

Study Design

The study was a prospective, randomised parallel group trial with 1-year follow-up. Comparison was made between an AHR strategy to reduce mannitol AHR to a PD_{10}≥635mg by serially incrementing the ciclesonide dose every 2 months according

to a predefined dosing regimen, with a control strategy based on the British Thoracic Society guidelines for asthma management. Venous blood samples for markers of bone turnover were taken at baseline (end of ICS step down) and at the final 12-month visit along with overnight 10-hour urinary cortisol (corrected for creatinine) at the same time points.

Measurements

Standardised spirometry was performed to measure FEV_1 using MicroMedical SuperSpiro (Carefusion, Basingstoke, UK)[216]. Mannitol challenge was measured as described previously with dry powder mannitol challenge (Aridol Pharmaxis Ltd, Sydney, Australia), to determine the PD_{10} [217]. Assessment of suppression of endogenous cortisol through systemic absorption of ICS was made by quantifying overnight 10hour urinary free cortisol corrected for creatinine (OUCC), this has been shown to be as sensitive as an integrated 24hour serum or urine profile[296]. Furthermore, OUCC was found to be more sensitive at detecting adrenocortical suppression in response to high dose ICS than low dose ACTH stimulated cortisol[297]. Assessment of bone formation was made using serum amino-terminal propeptides of type I and III collagen – PINP and PIIINP respectively. Bone resorption was assessed using serum pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen (ICTP), and type I collagen cross-linked C-telopeptide (CTx). Approximations of bone turnover were made using PINP/ICTP and PIIINP/CTx ratios.

All assays were performed in duplicate in a blinded fashion in the same laboratory. Urinary free cortisol was measured using a commercial radioimmunoassay kit (DiaSorin Ltd, Wokingham, Berkshire, UK). The intra-assay coefficient of variation

(Cv) was 21% with the inter-assay Cv 13%. This assay has no cross-reactivity with ciclesonide or des-CIC. Urinary creatinine was measured using the RATE technique on a Cobas-Bio auto analyser (Roche Products, Welwyn Garden City, UK) with an inter-assay Cv of 4.3%. PINP, PIIINP and ICTP were measured using commercial radioimmunoassay kits (Oxford Biosystems, UK) with inter/intra-assay Cvs 1.6% / 6.0%, 4.6% / 7.9%, and 9.3% / 5.4% respectively. CTx was measured using an ELISA assay (Immunodiagnostics, UK) with inter/intra-assay Cvs 24% / 21%.

Statistical analysis

Data were initially checked for normality prior to analysis, with non-Gaussian data logarithmically transformed to achieve normality. Post hoc calculations demonstrated that our sample size (50 patients per independent group) provided 81% power (two-tailed) to detect a minimal important difference of 30% in the CTx bone resorption marker between groups with a SD = 52% and α-error 0.05. Comparisons within and between groups for baseline and 12 month values and between baseline and 12 months were made using Student's t-test for paired and independent groups respectively (two-tailed), with significance set at $p<0.05$. The between group comparisons (i.e. AHR vs. Control) were considered as being of primary interest and the within group (i.e. baseline vs. 12 months in either AHR or Control) as secondary.

RESULTS

119 patients completed the reference study. Of these, 100 patients had suitable blood samples stored for analysis of bone markers after the trial. This comprised 50 patients in each of the AHR and Control groups. The baseline demographics for both groups can be seen in Table 21, demonstrating they were well matched with no significant differences at baseline between the two groups.

Table 21. Baseline Demographics.

Baseline Variable	Control (n=50)	AHR (n=50)	p-value
Age (years)	54.4 (51.2, 57.5)	53.8 (50.6, 57.0)	0.79
Gender (M:F)	28 : 22	27 : 23	0.84
FEV_1 (L)	2.60 (2.40, 2.79)	2.56 (2.38, 2.73)	0.78
FEV_1 (% predicted)	88.9 (84.5, 93.3)	85.8 (81.6, 90.0)	0.31
Mannitol PD_{10} (mg)*	96.0 (64.6, 142.8)	107.4 (73.7, 156.6)	0.69
PINP (µg/L)*	38.4 (33.9, 43.4)	39.0 (34.7, 43.9)	0.85
PIIINP (µg/L)*	2.93 (2.66, 3.21)	3.07 (2.71, 3.47)	0.55
ICTP (µg/L)*	2.54 (2.32, 2.78)	2.71 (2.42, 3.02)	0.39
CTx (µg/L)*	0.16 (0.13, 0.20)	0.15 (0.12, 0.18)	0.68
PINP/ICTP ratio*	15.1 (13.0, 17.5)	14.4 (12.5, 16.7)	0.66
PIIINP/CTx ratio*	18.6 (14.5, 23.8)	20.3 (15.7, 26.3)	0.63
OUCC (nmol/mmol)*	7.28 (5.91, 8.96)	7.44 (6.28, 8.81)	0.87
CIC dose (µg/day)	138	155	0.33

Data presented as arithmetic mean (95% confidence intervals) unless stated. *Geometric mean (95% confidence interval). AHR = Airway hyper-responsiveness. FEV_1 = Forced expiratory volume in 1 second. PD_{10} = Provocation dose (mannitol) causing 10% fall in FEV_1. Serum bone formation markers = PINP & PIIINP. Serum bone resorption markers = ICTP & CTx. OUCC = Overnight urinary cortisol/creatinine ratio. CIC = Ciclesonide dose (ex-actuator).

The final mean doses of ciclesonide (ex-actuator) after 12 months in each group were 202µg/day for controls and 507µg/day for AHR group, $p<0.00001$. This mirrors what was found in the reference study. The ICS dose increments over the 12-month study period for each group are illustrated in Figure 25, where it can be seen that even after

just 2 months, the AHR patients were receiving significantly greater amounts of ciclesonide, with the gulf between doses continuing to widen over the course of the year.

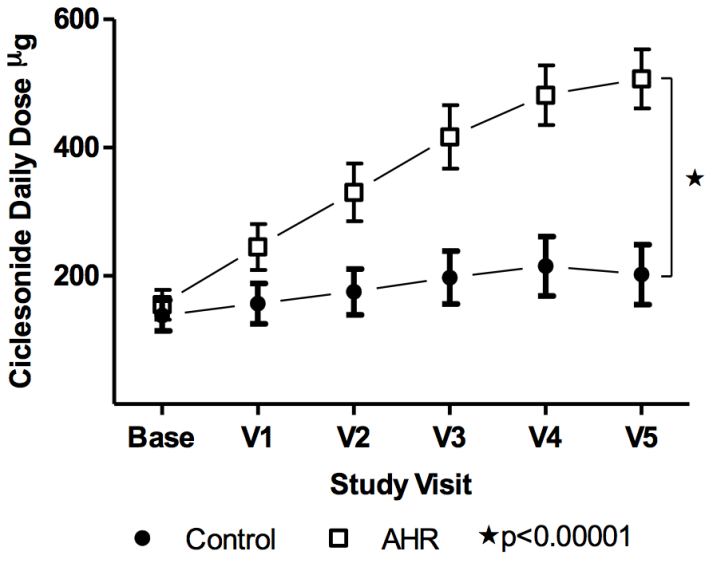

Figure 25. Ciclesonide titration over 1 year.

Incremental titration of ciclesonide (as ex-actuator dose) between groups at 2-month intervals over 1 year. Data presented as arithmetic means with 95% confidence interval error bars. AHR = airway hyper-responsiveness.

For the primary study comparison between the two groups, there were no significant differences after 12 months for markers of bone formation: PINP and PIIINP (Figure 26a), nor for markers of bone resorption: ICTP and CTx (Figure 26b). The bone turnover ratios PIIINP/CTx and PINP/ICTP at baseline and study end were also not significantly different between groups and are illustrated in Figure 26c and 26d respectively.

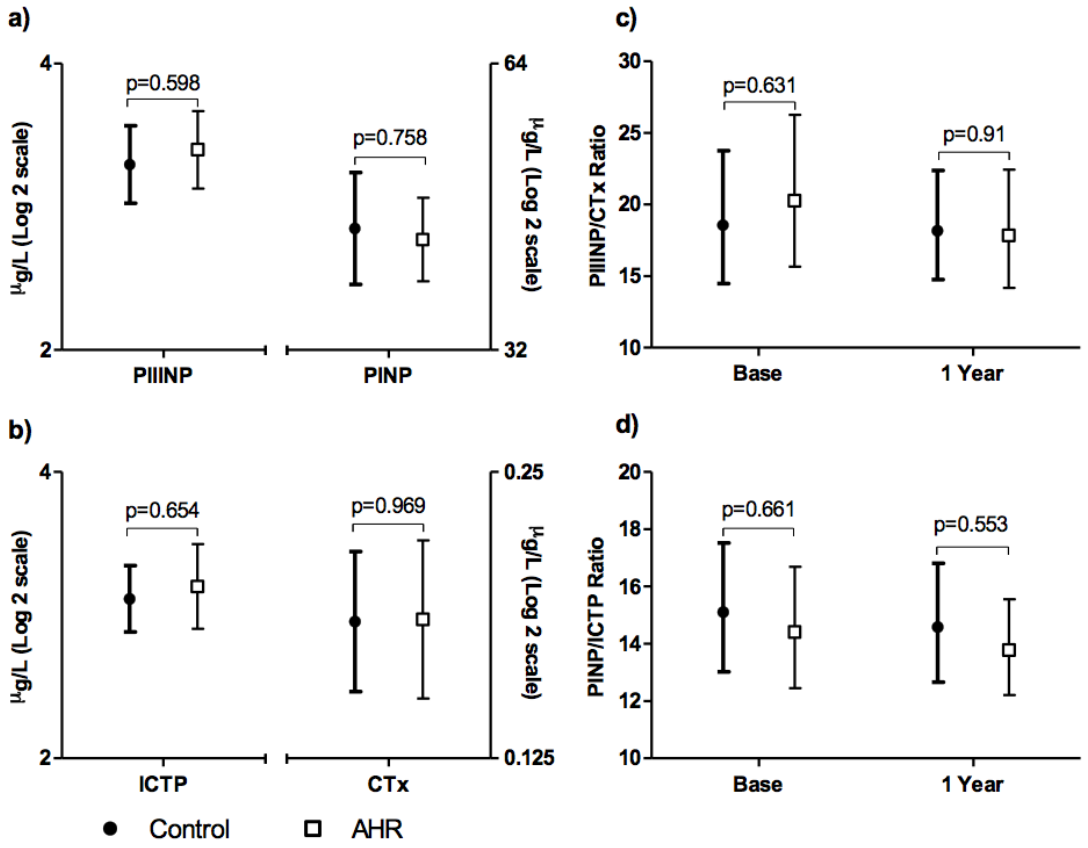

Figure 26. Bone turnover markers and ratios at 1 year.

Bone turnover markers at 12 months (a, b) and bone turnover ratios at 0 and 12 months (c, d) between groups. Data presented as geometric means with 95% confidence interval error bars. Bone formation markers (a) PINP & PIIINP = serum amino-terminal pro-peptides of type I and III collagen respectively. Bone resorption markers (b): ICTP = serum pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen; CTx = type I collagen cross-linked C-telopeptide. Bone turnover ratios: PIIINP/CTx (c) and PINP/ICTP (d). AHR = Airway hyper-responsiveness.

Figure 27 confirms that the lack of difference in OUCC found in the reference study between groups at baseline and study end is mirrored in this subgroup of patients, indicating no significant HPA axis suppression in either group. This is further confirmed on Figure 28 showing all the actual OUCC values at baseline and 12 months for both groups, compared to a threshold of 3nmol/mmol – above which adrenal function is considered to be normal[226,227]. Reassuringly, the few patients below this threshold at baseline improved their adrenal function during the trial in both groups.

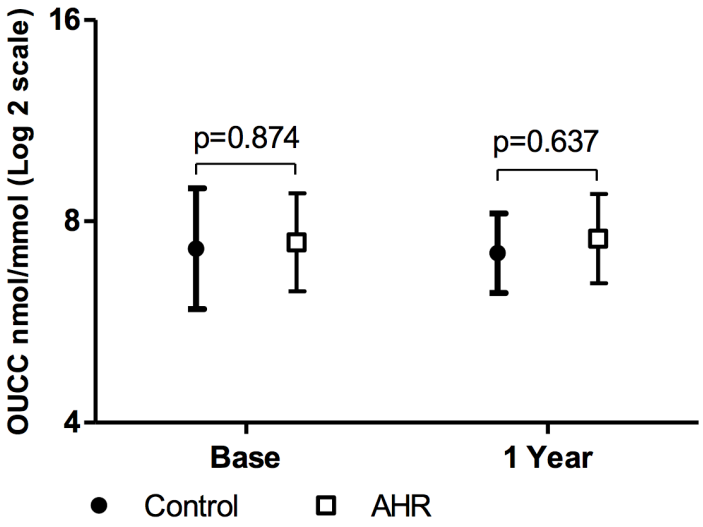

Figure 27. OUCC between groups at baseline and 1 year.

Data presented as geometric means with 95% confidence interval error bars. OUCC = Overnight urinary cortisol/creatinine ratio. AHR = Airway hyper-responsiveness.

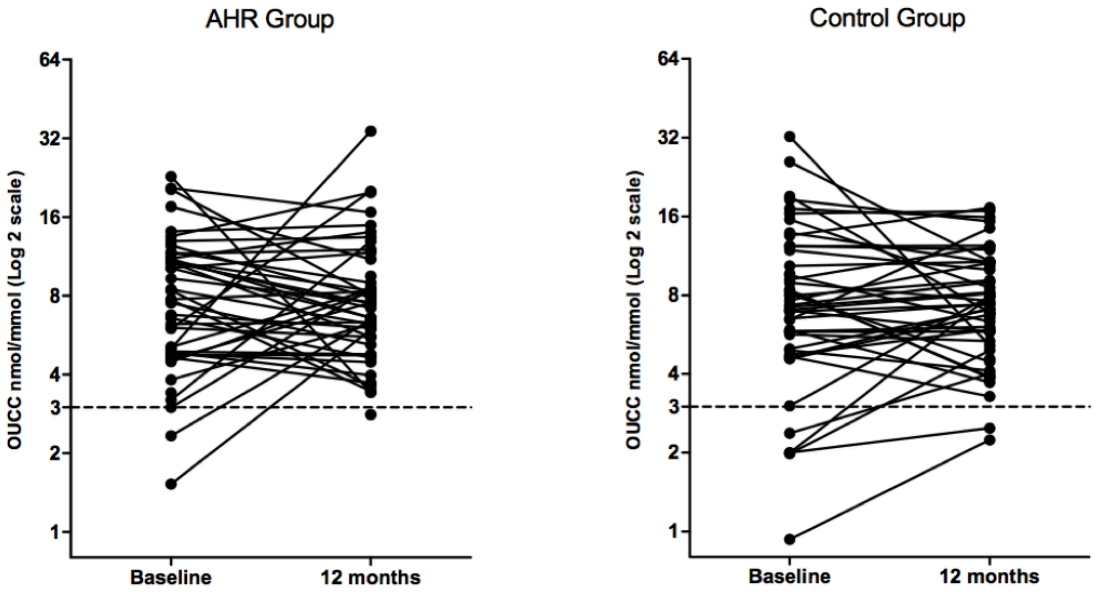

Figure 28. Scatterplots for individual OUCC ratios.

For both the Airway Hyperresponsiveness (AHR) and Control groups, absolute values are displayed for each individual at baseline and joined by a solid line to their respective values at 12 months. The interrupted line represents the cut-off value above which the overnight urinary cortisol/creatinine (OUCC) ratio is considered to be normal.

Results for bone markers and bone turnover ratios at baseline and at 12 months within both groups can be seen in Table 22.

Table 22. Serum bone markers and ratios at baseline and 1 year.

	Control			AHR		
Variable	**Baseline**	**1 year**	**P-value**	**Baseline**	**1 year**	**P-value**
PINP (µg/L)	38.4 (33.9, 43.4)	42.9 (37.5, 49.2)	0.01	39.0 (34.7, 43.9)	41.8 (37.8, 46.3)	0.13
PIIINP (µg/L)	2.93 (2.66, 3.21)	3.13 (2.85, 3.44)	0.02	3.07 (2.71, 3.47)	3.25 (2.96, 3.57)	0.20
ICTP (µg/L)	2.54 (2.32, 2.78)	2.94 (2.72, 3.19)	<0.001	2.71 (2.42, 3.02)	3.03 (2.74, 3.36)	0.04
CTx (µg/L)	0.16 (0.13, 0.20)	0.17 (0.15, 0.21)	0.41	0.15 (0.12, 0.18)	0.17 (0.14, 0.21)	0.11
PINP/ICTP ratio	15.1 (13.0, 17.5)	14.6 (12.7, 16.8)	0.50	14.4 (12.5, 16.7)	13.8 (12.2, 15.6)	0.38
PIIINP/CTx ratio	18.6 (14.5, 23.8)	18.2 (14.8, 22.4)	0.81	20.3 (15.7, 26.3)	17.8 (14.2, 22.4)	0.32

Data are presented as geometric mean (95% confidence interval). Serum bone formation markers = PINP & PIIINP. Serum bone resorption markers = ICTP & CTx.

Percentage changes from baseline for bone markers and ratios within each group are recorded in Table 23.

Table 23. Comparison of within group changes in bone markers/ratios over 1 year.

Variable	Control (%)	AHR (%)	p-value
PINP (µg/L)	10.6 (2.6, 17.9)	6.1 (-3.3, 14.8)	0.50
PIIINP (µg/L)	6.6 (1.5, 11.4)	6.7 (-1.9, 14.6)	0.93
ICTP (µg/L)	13.7 (7.1, 19.7)	10.8 (0.7, 19.8)	0.62
CTx (µg/L)	9.0 (-13.6, 27.1)	14.8 (-3.1, 29.6)	0.66
PINP/ICTP ratio	-3.5 (-14.4, 6.3)	-4.3 (-13.4, 5.3)	0.89
PIIINP/CTx ratio	-3.0 (-31.1, 19.0)	-11.1 (-29.3, 10.6)	0.61

Data are presented as percentage change from baseline (95% confidence interval). Serum bone formation markers = PINP & PIIINP. Serum bone resorption markers = ICTP & CTx.

Whilst there were statistically significant increases over 12 months for PINP, PIIINP and ICTP within the control group, these did not reach the minimal important difference (~30%) to exclude biological variability, i.e. 10.6%, 6.6% and 13.7% respectively. There was also a statistically significant increase in ICTP (10.8%) in the AHR group without reaching the minimal important difference. Moreover all differences as change from baseline for bone markers and ratios were not statistically different when comparing between the groups over the 12-month period.

Individual within group percentage changes from baseline for bone formation (PINP) and resorption (ICTP) in both groups are represented using scatterplots with superimposed mean ± 95% confidence intervals and representative 30% minimum clinically important difference (MCID) cut-offs for both in Figure 29.

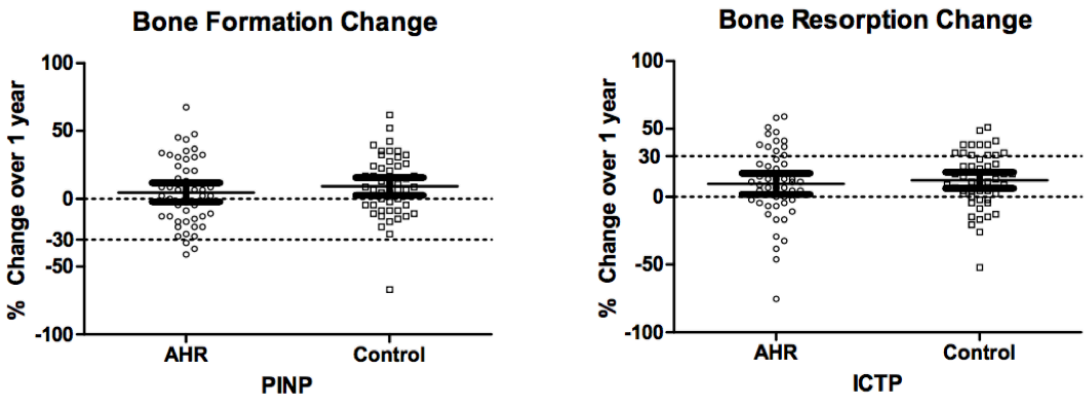

Figure 29. Individual changes in markers of bone formation and resorption.

Scatterplots of percentage changes for each patient between baseline and 12 months with superimposed mean ± 95% confidence intervals and representative -30% cut-off for bone formation and +30% cut-off for bone resorption (interrupted lines) as minimal clinically important differences.

DISCUSSION

The results of the present study show that in patients exposed to ciclesonide over 12 months, using a higher dose to achieve better asthma control has no adverse effects on sensitive biochemical markers of bone turnover or on adrenocortical function. It has long been recognised that systemic corticosteroid therapy leads to a rapid reduction in bone formation markers, such as osteocalcin – a marker of osteoblast activity, leading to reduced bone mineralisation and ultimately bone loss[117,118,298,299]. The role of markers of bone resorption has been less clear in this regard[117,298,299] but seem more useful for monitoring anti-resorptive therapy such as the bisphosphonates[300].

More sensitive biomarkers of bone metabolism – the collagen peptides - have emerged in recent years. These markers can be measured in the serum or urine, however, it has been demonstrated that markers in serum are more sensitive in terms of signal-to-noise ratio than their urinary counterparts with the added benefit that they do not require correction for the patient's glomerular filtration rate[300]. One example of this is the carboxy-terminal collagen crosslinks or CTx. This crosslink peptide sequence of type I collagen is the part cleaved by osteoclasts during bone resorption, reflecting osteoclastic activity[301]. CTx has been shown to more accurately track changes in levels of bone resorption after bisphosphonate therapy than other markers such as urinary N-telopeptide crosslinks[302]. This is aided by its low coefficient of variability (15.1% over 3 months) and large changes in response to bisphosphonate therapy[302]. Estimates of the MCID for CTx do vary, i.e. the degree of change that can exclude normal biological variability. It has been suggested that for a coefficient of variation of 10%, a change of at least 25-30% must be observed for serum markers to consider significant evolution[300]. Other papers have quoted 30.2%[302] and as much as

55-60% for change in CTx to be clinically significant[303]. Indeed, when it comes to predicting fracture risk, an elevated CTx is associated with an increased risk of osteoporotic fractures[304,305]. A cut-off of 30% change has been suggested elsewhere, i.e. a >30% fall in CTx with bisphosphonate therapy reduced fracture risk[306]. We have taken a 30% change as the MCID on which to base our post hoc power calculation. In addition to CTx we have used other serum biomarkers to reflect both bone formation (PINP & PIIINP) and resorption (ICTP) in a novel way in persistent asthmatics receiving both high and low doses of the inhaled corticosteroid ciclesonide. In one study, PINP was investigated as a marker of raloxifene therapy in post-menopausal women who had evidence of osteoporosis[307]. PINP reductions were 11% with placebo and 41% with raloxifene. The reduction in the raloxifene group accounted for 28% of the total reduction in vertebral fracture risk. This again suggests that a MCID in these markers is around 30-40%.

There have been numerous studies demonstrating both short and long term effects of ICS on the HPA axis[293,297,308,309], with some types of ICS more potent in their suppression of it as mentioned previously[290]. This logically leads one to consider the consequent effect on bone turnover and potential for the development of osteoporosis. Some studies have indeed shown deleterious effects on markers of bone turnover and bone mineral density with ICS[119], with some doubt cast on this by other longer term studies failing to demonstrate any effect on bone with high dose ICS with or without significant HPA axis suppression[120,121], alluding to the possibility of a disconnect between the two. We hypothesised that if such a disconnect is present, then it may be possible that one may have a normal HPA axis yet still suffer deleterious effects on bone turnover during long-term ciclesonide therapy.

Reassuringly, our study suggests that even with long-term treatment using a higher dose of inhaled ciclesonide - both sensitive markers of bone formation PINP and PIIINP, as well as of bone resorption ICTP and CTx, are no different between groups to any clinically significant degree at the end of a 1-year period. This is also in accord with the lack of any deleterious effect on the HPA axis as measured by OUCC. What is most relevant is the lack of any reduction in bone formation because this is where most effect is seen with oral systemic corticosteroid therapy, i.e. PINP and PIIIP levels remain approximately the same over the 12 month period in the higher ICS dose AHR group. For PINP only 3 patients in the AHR group fell below the -30% MCID, at -32%, -37% and -41%, which is reassuring particularly as we had considered using a more conservative MCID of -40%. There was one significant outlier below -30% in the control group for PINP, but all other patients remained above the line in this group. In fact, bone formation markers increased overall in the control group, possibly suggesting an effect of higher ICS dose with the lack of increase in the AHR group, but as mentioned above, these increases did not meet our assumed MCIDs. There were statistically significant increases in the ICTP resorption marker in both groups to an approximately equal degree, without meeting the MCID; but no significant increases in CTx which may confirm that these measure different aspects of bone resorption. For ICTP, there were 12 patients in the AHR groups and 13 patients in the control group where their change was greater than the 30% MCID. These numbers reduced to 7 for AHR and 3 for control when utilising a 40% cut-off. Reassuringly there were similar numbers of patients who had an increase above 30% in bone formation (PINP) – 11 patients in AHR group and 10 in the control group. We also approximated overall bone turnover with two ratios of bone formation to bone resorption – PINP/ICTP and PIIINP/CTx. Once again these showed no

significant change over a year either within or between groups, further confirming the safe therapeutic profile of ciclesonide at higher doses.

The most obvious limitation of this study is the lack of confirmatory evidence for our findings through formal measurement of each patient's bone mineral density at beginning and end. However, biomarkers of bone turnover have shown a strong correlation with radiological bone density measurements[310,311]. Another potential drawback of this data is the fact that ciclesonide does not tend to cause much adrenal suppression in general, compared to fluticasone propionate, for example. The strengths of this study include the prospective nature of data collection in a community setting, the long treatment period of a year, and the statistical power of the study with large patient numbers to detect significant changes in the bone markers. Even though this was a subgroup of the original study, our findings mirror what was found in that study in terms of lack of OUCC suppression, helped by the fact that we studied 84% of the original participants who had suitable blood samples for analysis.

In conclusion, we have demonstrated that the higher doses of inhaled ciclesonide required to achieve superior asthma control through reducing levels of airway inflammation over a 1 year period do not adversely affect sensitive biomarkers of bone formation, resorption and their respective turnover ratios in persistent asthmatics, as compared to a lower dose of inhaled ciclesonide. Moreover, this is in keeping with lack of HPA axis suppression in these patients, providing reassurance that inhaled ciclesonide has a safe systemic therapeutic profile even with the higher dosing required to achieve better asthma control.

CHAPTER 9:

DISCUSSION AND CONCLUSIONS

DISCUSSION

This body of work incorporates a variety of areas of study with the aim of furthering our understanding of how we might better individualise the mainstay of asthma controller therapy for patients, namely inhaled corticosteroids (ICS), by examining ICS dose responses to multiple asthma outcomes that delineate the disease. This thesis therefore begins by exploring both intrinsic and extrinsic factors that may alter or help predict responsiveness to incremental ICS dosing. The utility of pharmacological manipulation of the ICS dose response with an additional therapy is then examined for any steroid sparing effects in this regard, and to provide additional insight into the underlying pathophysiological mechanisms. Further, the relationships between inflammatory and other asthma outcomes are examined both when patients are receiving static doses of ICS, as well as following incremental ICS dosing. Finally, potential systemic effects of this degree of ICS dose ramp are examined in a long-term study of sensitive markers of bone turnover where patients have had their ICS titrated against either standard care (BTS guidelines) or inflammation driven AHR.

Current ICS titration strategies for the treatment of asthma are largely based on guidelines produced from professional groups such as the British Thoracic Society[10]. Whilst they incorporate an extensive overview of asthma management, when it comes to the detail of incremental ICS dosing, any titration is based on simple measures applicable in the community including symptoms, basic pulmonary function measurements, and beta-2 agonist reliever use. This is certainly appropriate when we consider the asthmatic population as a whole, given that most will have mild to moderate disease where low doses of ICS can easily correct these parameters in most

cases. However, this overarching plan can begin to unravel in several ways for individual patients. Either the patient or physician may not strictly adhere to treatment guidelines, given that we know strict guideline adherence in a clinical trial improves asthma outcomes[32]. There is also an ongoing attendant morbidity and mortality[25] with asthma that is theoretically avoidable, and proves that this disease is being significantly undertreated in certain individuals for a variety of potential reasons. Furthermore, patients are not good at recognizing when their disease might be uncontrolled[30] and therefore don't seek additional help. Pointedly, it is impossible to know if there is any ongoing inflammation or airway hyper-responsiveness (the two main hallmarks of the asthmatic disease process) without specifically testing for them – which currently is prohibitive in a community setting. However, given ongoing asthmatic inflammation and AHR are precursors of poor future asthma control and exacerbations[46,152,155], it is logical that one would wish to know about them before completing any individual's ICS treatment review. It is this inability to completely quantify any future risk that leaves us in a precarious position. Even well validated, yet relatively simple, measures of asthma control such as the asthma control questionnaire[33] are still not well utilised for this purpose. It is therefore imperative that we improve our understanding of incremental ICS dose responses, not only to usual measures of symptoms and pulmonary function, but also to measures of the underlying ongoing disease process, specifically inflammation and AHR.

The first study in this thesis began with the evaluation of ICS dose response on FeNO levels. This was a phenotype driven study, where asthmatic patients were selected on the basis of displaying high FeNO levels from the outset following ICS washout. The reason for this study design was to observe how predictable their ICS dose response

might be, both in relation to FeNO, as well as on other clinically relevant measures of inflammation and asthma control. Furthermore, whilst a dose response of FeNO to ICS had been observed previously[159], we decided to examine this relationship using domiciliary FeNO measurements – a technique used before in children, but not in adults with asthma. We believed this method of serial diurnal FeNO measurement would also improve the signal to noise ratio of this outcome measure.

We learned a number of things about how domiciliary FeNO responds to ICS from this study. Firstly, we observed significant improvements in both morning and evening FeNO levels with both low and high dose fluticasone, with significant dose separation between the two domiciliary response curves. Furthermore the domiciliary measurement approach helped us to see that improvements in FeNO levels follow a one-phase exponential decay pattern, with 50% reduction in FeNO levels seen after only 2.9 or 2.3 days (for 100µg/day and 500µg/day FP doses respectively, which equate to approximately 200µg and 800-1000µg BDP equivalent doses). A rapid rise in FeNO levels back to baseline levels also occurred during ICS-free washout periods of only 2 weeks. FeNO is therefore very sensitive to the effects of ICS, even at low doses, confirming previous findings in this regard[220]. It may, therefore, be useful as a rapidly responsive measure of compliance to ICS therapy. Indeed, it has been shown as a useful measure of non-adherence to treatment in severe asthmatics[158].

The most clinically relevant finding from this study is the relationship of FeNO response to ICS leading to significant improvements in measures of asthma control and quality of life. Not only were significant improvements seen over such a short period of time, 2 weeks, but these improvements were clinically relevant with mean

changes of >0.5 for both ACQ and AQLQ scores, the minimum clinically important difference for both these outcome measures[33,166]. Indeed, ACQ improved to a score of <0.75 with both doses of FP indicating well-controlled asthma[34]. In addition to the correlation of FeNO improvement with measures of asthma control and quality of life, the fall in FeNO with ICS was followed by improvements in other measures of asthmatic inflammation. However, the same degree of dose separation was not seen equally between these other inflammatory outcome measures, suggesting a further level of complexity to the airway inflammation story. That is, serum ECP required higher ICS dosing to be suppressed, whereas mannitol challenge and blood eosinophils improved initially with low dose ICS, but not significantly further with the higher ICS dose. These findings could be due in part to the fact that the study was not powered on these as primary outcome measures, or that treatment for 2 weeks was simply too short a time period to observe any meaningful dose separation in these other outcomes. However, the fact that we still observed meaningful improvements in asthma control, might mean that domiciliary FeNO measurement is more sensitive in this regard. There were statistically significant improvements in FEV_1 as well, but these did not meet the minimum clinically important difference for this outcome measure, perhaps due to patients having relatively preserved spirometry from the outset. Future studies using domiciliary FeNO measurement should focus on longer-term outcome measures such as exacerbations, as well as examining for any improvement in compliance to ICS therapy by perhaps using daily domiciliary FeNO measurement to help reinforce the need for this important treatment for individual patients.

Having examined the ICS dose response of FeNO as an intrinsic factor related to asthma control, the second study in this thesis examined the relationship of obesity to ICS dose response as an extrinsic factor that may influence this. Previous studies had suggested both possible blunting of glucocorticoid responsiveness[181] and indeed reduction in overall asthma control in obese compared to normal weight asthmatics[180], when compared using static doses of steroid. They did not however examine any dose response relationship. This thesis' study was a post-hoc analysis of data from a pharmacodynamic bioequivalence study comparing dose ramps between 200µg/day – 800µg/day of both CFC and HFA-budesonide. We elected to study the HFA-budesonide arm as it was more clinically relevant to today's practice now that CFC-propelled inhalers are obsolete. There was no particular primary outcome measure overarching the study, however, patients were selected on the basis of the presence of AHR to methacholine with a PC_{20}<4mg/ml.

Once separated into normal weight (BMI<25kg/m^2) and overweight (BMI≥25kg/m^2) groups, we found greater improvements in both FeNO and symptom responses to incremental ICS dosing in the normal weight group, where no differences were seen for either AHR to methacholine or FEV_1 between BMI groups, despite significant overall improvements with ICS for both these outcomes. As with the FeNO study, most improvement was seen with low dose ICS for both FeNO and symptoms. Whilst baseline FeNO levels were different between the BMI groups, i.e. higher in the normal weight group, baseline symptom scores were not different, yet followed a remarkably similar pattern of response to FeNO between BMI groups following incremental ICS dosing. It might be, therefore, that FeNO is a more sensitive marker when it comes to following symptom improvement in persistent asthma, than AHR or

FEV_1; albeit baseline lung function in this study was once again relatively preserved from the outset. This putative further room for improvement in overweight asthmatics for FeNO and symptoms may also be important when it comes to exacerbation risk, given that pulmonary function is not such a good predictor in this regard as seen in previous studies using inflammation or AHR to guide steroid therapy in asthma[6,13,14].

These findings generate several hypotheses to try to explain the differences in outcome measures and ICS dose responses between the BMI groups. Given that we have classified obesity as an extrinsic factor here, it raises the possibility that altered lung mechanics[239,240] from increased chest wall restriction or increased elastic recoil might be the reason for lack of symptom improvement in the overweight group upon incremental ICS dosing. To put it another way, perhaps the obesity itself is adding to the symptom burden, independent of any asthmatic symptoms, and so would not respond to ICS in the same manner. Whilst an attractive proposition, we did not observe any spirometric restriction in the overweight compared to normal weight group. Furthermore, the overweight group was certainly not morbidly obese, with a mean BMI of $30kg/m^2$. It could be, therefore, that symptoms are more closely related to airway inflammation and small airways function rather than large airway calibre. We did not, however, specifically measure small airway function in this study. Another potential theory is that of reduced (distal) lung deposition of ICS particles in the overweight group, leading to reduced efficacy for FeNO and symptoms. This is backed up by the fact that FeNO is a whole lung measurement (from both large and small airways); whereas FEV_1 and AHR relate to central large airway responses in the main. We also found a trend to greater cortisol suppression in the normal weight

group; again alluding to possible reduced systemic absorption (from reduced lung delivery) in the overweight group. This could also be due to reduced glucocorticoid sensitivity[181], but we did not have the kinetics data to confirm or refute this. Future prospective studies to answer this question could be performed using imaging studies of lung deposition and small airway measurements, using both small (MMAD<2μm) and large particle (MMAD>2μm) ICS compared across a wider range of BMI.

If FeNO and symptoms are less sensitive to ICS in overweight and obese asthmatics, then patients may require their ICS doses to be titrated further to gain the same response as in normal weight individuals. This is relevant, given that control of inflammation can be more beneficial in preventing future exacerbation risk[14,220], over and above simply normalizing symptoms or lung function. Alternatively, it has been shown that the addition of other therapies such as leukotriene receptor antagonists might be more beneficial earlier in the asthma treatment ladder for overweight asthmatics, as their effects have been shown to be upregulated by leptin, which is more prevalent in obese asthmatics[248,249]. It is ultimately important to remember, however, that achieving weight loss in these individuals will not only benefit their asthma[182], but will also prevent many other co-morbid sequelae such as diabetes and cardiac disease.

The third study presented in this thesis investigated the role of propranolol (a non-cardioselective beta-blocker and inverse agonist at the beta-2 adrenoceptor, B2ADR[262]) as a novel, counterintuitive, corticosteroid-sparing agent in persistent asthma. Previous murine and open-label human studies have shown that another B2ADR inverse agonist, nadolol, significantly improved AHR when patients were

steroid naïve[252-256], as well as potentially acting as a steroid-sparing agent[260]. In order to answer this question, we utilised a positive control arm of higher ICS dosing compared to low dose ICS plus propranolol, measuring the primary outcome of change in histamine AHR compared to low dose ICS alone between both treatment arms. Furthermore, we wished to examine for any possible anti-inflammatory effects of propranolol as a secondary outcome, again with the benefit of the higher dose of ICS as a positive control. Safety was clearly paramount in this study; indeed it required careful and detailed discussions with the local ethics committee in order for them to provide their approval, including conceding that we would put 'risk of death' in the participant information sheet! We therefore monitored a variety of safety measures in domiciliary fashion throughout the study for each patient. This was perhaps even more important given that patients enrolled in the study could be considered to have moderate severity of asthma, as evidenced by almost half being on combination ICS/LABA therapy pre-study, with a mean pre-study ICS dose of 406μg/day (95% CI: 243, 569) for the whole group, yet still displaying moderately severe AHR to histamine with mean PC_{20} 1.39mg/ml (95%CI: 0.93, 2.07) as well as moderately elevated FeNO levels 40ppb (95%CI: 29, 55).

We found that the addition of propranolol to low dose ICS did not alter AHR to histamine, despite observing significant systemic effects of beta-blockade on heart rate and blood pressure measurements. Moreover, the lack of any steroid-sparing activity of propranolol on histamine AHR was confirmed by the higher dose of ICS alone leading to further improvement in histamine AHR with a mean improvement of more than 1 doubling dilution difference over baseline, i.e. meeting the pre-specified power calculation. In addition, propranolol did not improve any inflammatory

measures either, whereas the higher ICS dose significantly further improved all measured inflammatory outcomes including FeNO, blood eosinophils and serum ECP. Small but significant improvements in symptoms were also seen in the positive control arm, but there were no improvements in measures of lung function despite the higher ICS dosing. Reassuringly, we did not observe any clinically significant adverse pulmonary effects in the propranolol arm. There was some mild blunting of salbutamol recovery post-histamine challenge, as well as a reduction in evening, but not morning, domiciliary FEV_1. Importantly, while asthma control (as measured by ACQ) did not improve significantly with higher dose ICS (from a well-controlled baseline), neither did it worsen upon chronic exposure to 80mg/day of sustained release propranolol.

Inverse agonist activity occurs at the B2ADR in the presence of certain beta-blockers including propranolol and nadolol, essentially 'switching off' the receptor by reducing basal constitutional activity[262,263] with the potential, therefore, to up-regulate other B2ADRs. Indeed B2ADR upregulation was seen in a previous pharmacological study after only 2 days of propranolol exposure with a fully restored isoprenoterol-induced cyclic-AMP response at 2 weeks[266]. We were therefore able to cover the first period of any potential dramatic worsening in bronchoconstriction with tiotropium (a long-acting muscarinic antagonist, LAMA), both to allow time for the B2ADRs to upregulate, and to prevent unopposed acetylcholine-induced bronchoconstriction due to beta-2 receptor blockade with propranolol[261]. Moreover, we utilised a gradual dose ramp of propranolol, in order to further prevent any associated initial worsening of airway bronchoconstriction. We showed that both of these safety strategies were successful in our study.

However, with regard to the lack of improvement in AHR with the addition of propranolol to low dose ICS, it is possible that any clinically significant upregulation of B2ADRs had already been achieved with the low dose of ICS alone[86-88], negating any effect the propranolol might have had. Nevertheless, the higher ICS dose still achieved a greater improvement in histamine AHR, confirming that the pharmacological effects of ICS are much more wide-ranging than simply upregulating B2ADRs, e.g. through their effects on suppressing inflammation. It is perhaps unsurprising that there was little improvement in both ACQ and lung function with higher dose ICS, given that patients started out with relatively normal baseline levels of both when receiving low dose ICS. Nevertheless, it is more than a little worrying that we still observed significant consistent further improvements in both AHR and a variety of inflammatory markers with the higher ICS dose alone, that did not translate into observable improvements in either ACQ or lung function. The reason for this concern is due to previous studies showing that uncontrolled AHR and airway inflammation both independently predict a greater risk of future adverse events such as exacerbations[46,152,155], and this usually in the presence of preserved lung function, as in our study. It is perhaps more concerning that ACQ didn't change, and was even considered 'well-controlled' from the outset despite further room for improvement demonstrated for both AHR and inflammation upon higher ICS dosing. I say this because ACQ has itself been shown to predict future risk[29], and so its seeming normality here is perhaps falsely reassuring in this regard. A more interesting, but probably more dangerous study, would be to assess for any steroid-sparing effect of inverse-agonist beta-blockers in more severe asthmatics, where higher ICS dosing alone does not always achieve total asthma control; but there are already well established and probably safer additional therapies, such as add-on LABA therapy, for

this purpose. Ultimately, in this study of carefully monitored and well controlled persistent asthmatics, we have shown that propranolol can be given safely – which is an important message in terms of opening up this therapy to carefully selected asthmatics for its more usual indications, where previously non-cardioselective beta-blockers would have been denied.

The fourth study of this thesis examined the relationships between AHR to mannitol bronchial challenge, AHR to methacholine challenge, FeNO, salivary ECP and lung function as FEV_1; in asthmatic subjects receiving their usual doses of ICS. The reasons for exploring these were to increase the potential for wider utility of mannitol bronchial challenge testing by categorizing the severity of mannitol AHR into groups, and comparing these with established severity groupings for both methacholine AHR[20] and FeNO[212] in asthma. Given that mannitol challenge is a simple test to administer[209] (requiring only a spirometer and the ability to instruct the use of the dry powder inhaler delivery device), it is ideally suited to becoming established as a community, as well as a secondary care, method of additionally confirming or refuting the diagnosis of asthma[268], tailoring ICS therapy on the basis of mannitol AHR[6], or even predicting future risk of poor control and exacerbations – so called 'inflammometry'[14]. Furthermore, mannitol bronchial challenge reflects both airway hyper-responsiveness and airway inflammation at the same time, because it is an indirect challenge that invokes the inflammatory pathway in the airway mucosa to cause bronchoconstriction[269]; whereas direct bronchial challenge tests including methacholine only measure airway smooth muscle sensitivity. This means that it can measure both main hallmarks of the underlying asthmatic process simultaneously, potentially improving its diagnostic and monitoring efficiency further. We used

mannitol PD_{10} AHR levels rather than the standard mannitol PD_{15} values, both because we believed that we would find more responsive patients using this lower threshold, and perhaps more importantly because they were already being treated with moderate doses of ICS, thus we additionally wanted to improve the signal-to-noise ratio of the test in this setting. We showed that for those patients who were responsive at both PD_{10} and PD_{15} for mannitol, there was a very strong correlation between the two ($r=0.93$) confirming our proposition as valid.

This study revealed that by grouping mannitol AHR into mild, moderate and severe responses based on mannitol PD_{10} levels, these positively correlated with severity levels for both methacholine AHR and FeNO. Furthermore, when reciprocal correlations were examined using both methacholine and FeNO severity groups, the same correlations were observed for mannitol AHR. Moreover, correlations between all three outcomes persisted when examined as continuous rather than categorical variables. Indeed, clinically significant magnitudes of difference were identified between mild and severe mannitol AHR groupings for both methacholine PC_{20} and FeNO levels in these patients who were currently receiving ICS. There were no significant correlations, however, between any of these outcome severity groupings and FEV_1, salivary ECP or current ICS dose.

These findings confirm that mannitol bronchial challenge is a clinically useful measurement of both AHR and airway inflammation jointly. Indeed, mannitol AHR has previously been shown to have a similar sensitivity and specificity to methacholine challenge for diagnosing asthma[131,275]. Mannitol additionally has been shown to have a similar negative predictive value for excluding the diagnosis of

asthma as a FeNO<36ppb[275]. Also given its simplicity of administration, it could be a potentially invaluable tool for the diagnosis and ongoing management of asthmatic patients in a community setting; over and above the standard markers of symptoms and spirometry that are currently used for this purpose in primary care. Pointedly, the ability to measure multiple disease hallmarks at once, might improve our ability to better predict future adverse events such as exacerbations, but this requires further prospective study to confirm this hypothesis. It has again been shown here that FEV_1 does not reflect the underlying pathophysiological processes in asthma well, given the lack of correlation of this outcome with any of mannitol AHR, methacholine AHR or FeNO; albeit once again FEV_1 was relatively preserved in this patient cohort. Moreover, there was no correlation between current ICS dose and any of the severity groupings for the three outcome measures, perhaps telling us that these individual patients' underlying asthma severity had not been discriminated well enough to be translated into increasing ICS doses for patients with greater markers of asthma severity; or more simply perhaps that ICS compliance was not optimal. We did not formally measure asthma control in this cohort, but this would have been useful, particularly in relation to the question of optimal ICS dosing in the face of disparate asthma severity as demonstrated with three separate outcome measures.

The penultimate study presented in this thesis draws together the various strands of investigation in the previous studies by examining the dose responses of a variety of asthma outcome measures to a combination of commonly used ICS, utilizing similar dose ramps between 0, 200 and 800μg/day (BDP equivalent). The premise of this pooled analysis was to determine whether there might be any further room for improvement in any outcome measure beyond low dose ICS, in particular whether

there was any difference between routine measures of ICS dose response (symptoms and pulmonary function) and measures of the underlying pathophysiological processes in asthma (inflammation and AHR).

We found a plateau of the ICS dose response to both pulmonary function and symptoms beyond low dose therapy. However, with the same ICS dose ramp, we uncovered further room for improvement in measures of asthmatic inflammation (FeNO, serum ECP and blood eosinophils) and AHR (to both direct and indirect bronchial challenges). This disconnect, therefore, potentially challenges clinical equipoise, where mainly symptoms and pulmonary function are used to determine ICS response in both primary and secondary care[10]. Indeed, this leads to the potential for inadequate ICS dose escalation in an individual who presents with preserved spirometry and no significant symptoms, but has unrecognized ongoing underlying airway inflammation and AHR. Furthermore, given that patients do not always recognise when their asthma control is worsening[30], additional objective measurements of these two underlying pathological processes may help to achieve optimal ICS dosing, in order to prevent future exacerbations or further loss of asthma control[46,76,151].

There is therefore a growing body of evidence that points to the logical conclusion that we, as a community of asthma physicians, need to decide *how best* to include measures of asthmatic inflammation and AHR for routine asthma evaluation, rather than *whether* they should be included; not least because at the present time these are the best determinants of future risk in asthma that we have, and that by targeting these outcomes therapeutically, reductions in asthma exacerbations have been

achieved[6,13,14]. The simplest of these additional routine measurements might be blood eosinophil levels. Indeed, it has been shown that higher levels of blood eosinophilia >400 cells/μL accounted for more severe exacerbations and poorer asthma control, than in those asthmatics whose blood eosinophils are lower than this[77]. There was also a count-response relationship between blood eosinophil levels and asthma-related outcomes in that particular study. As previously discussed in this thesis, FeNO levels may also be useful as an additional measure in this regard[155]; and although expensive at the present time, the technology for FeNO measurement might become cheaper and more straightforward to administer in the future. AHR to mannitol bronchial challenge provides information about both hallmarks of asthma, namely inflammation and AHR simultaneously, and thus might provide a simple solution to increased community measurement of these outcomes[6] in addition to pulmonary function measurement and asthma symptom evaluation. Ultimately, it may be that we need a panel of tests[162] that cover the spectrum of asthmatic disease pathophysiology, in order to adequately personalise ICS therapy for an individual asthmatic. This has been partially achieved through the use of the asthma control questionnaire[33] that combines multiple objective facets of asthma symptoms and daily experience with measurement of lung function. Adding inflammatory/AHR measurements, perhaps with simple blood sampling for eosinophil levels to the ACQ for example, might be the first step in this process. However, these hypotheses would need to be validated in further ICS titration studies using future asthma control and exacerbations as strong and clinically relevant outcome measures.

The final study in this thesis rounds off the examination of the complete therapeutic profile of ICS dose response, by looking at the issue of safety when exposing patients

to higher doses of ICS in order to achieve optimal asthma control over a longer period of time. In particular this study focused on the most worrying long-term adverse effect of steroid therapy, namely alteration of bone metabolism that may ultimately lead to osteoporosis and, therefore, increased fracture risk. It is certainly clear that oral corticosteroids rapidly cause significant reductions in bone formation that lead to osteoporosis by tipping the balance of bone turnover to favour that of resorption with chronic steroid treatment[117,287]. Previous studies of ICS have been conflicting in this regard, albeit we can be assured of the safety of low to moderate doses. Indeed it is not until ICS doses are much higher (or more potent ICS are used such as fluticasone propionate), that biochemical effects on the HPA axis can be observed[49,290]. However, even when HPA axis effects are not seen, there may be a disconnect in regard to the potential for ongoing effects on bone turnover itself[120,121]. We therefore examined this hypothesis using ciclesonide (CIC) as the ICS, where CIC was titrated against either standard BTS treatment guidelines or using an AHR strategy resulting in higher ICS dosing in the AHR arm after the 1 year study period (mean doses 202μg/day vs. 507μg/day). We performed a post hoc analysis of previously stored, sensitive serum biomarkers of both bone formation and resorption, and used the same to approximate any differences in overall bone turnover between the groups comparing low and high dose ICS prospectively over a 1 year period.

We found no differences between the low (control) and high (AHR) dose ICS groups for either markers of bone formation (P1NP, P3NP), or bone resorption (1CTP, CTx) after the 1-year study period. Furthermore, ratios of bone turnover as approximated by P1NP/1CTP and P3NP/CTx were also no different between the two ICS doses after 1 year of treatment. These findings were additionally corroborated by observing

no deleterious impact on the patients' HPA axes, as measured by overnight urinary cortisol/creatinine ratio in either treatment group at the end of the 12 months. CIC is certainly well placed from the outset in this regard. Its pharmacological profile is such that very little is systemically absorbed due to 99% of it being protein bound[292]. It is perhaps therefore unsurprising that we saw no significant effects on sensitive markers of bone turnover with this particular ICS. Indeed, it may have been a different picture with a more systemically potent ICS such as fluticasone propionate[295]. Nevertheless, CIC was clearly able to achieve more optimal control of asthmatic inflammation as measured by mannitol AHR in the parent study with the higher ICS dose.

It is important to highlight the absence of any difference between ICS doses for the approximated ratios of bone turnover. While the mean differences in standalone bone markers were not different, some individual patients had significant increases in bone resorption markers (1CTP). It is difficult to be certain of the MCID for these markers, but it is considered to be around 30%. With some individuals falling outside this threshold, it is reassuring that their overall bone turnover ratios remained unchanged.

The major limitation of this study was a lack of confirmatory bone densitometry measurement at either end of the study period, particularly given the mean age of all patients being 54 years. However, the serum biomarkers used here were more sensitive than bone densitometry, and have also been shown to strongly correlate with these radiological measurements[310]. Indeed, the serum bone markers are also more sensitive and reliable than their urinary counterparts, with a lower Cv and without the need for correction with renal function. The particular strengths of the study are both

the long study period of 1 year, as well as the prospective nature of bone marker measurement throughout the study.

Overall, therefore, we can be assured that by embarking on a strategy of total asthma control using the higher doses of ICS required to dampen the underlying inflammatory process (as measured here using mannitol AHR), there is unlikely to be any significant detrimental long term effect on bone metabolism.

To complete this discussion, it is important to identify future areas of research that should be considered on the basis of the findings from this thesis. I believe the main finding that requires more work, is the identification of asthmatic patients who may have preserved symptoms and lung function, but ongoing underlying and unrecognised airway inflammation and AHR. This is because the presence of both of these may lead to worsening asthma control or exacerbations in the future. This therefore suggests that we need to be measuring multiple facets of asthma pathophysiology in order to best predict future outcome, and therefore the most appropriate treatment for an individual asthmatic. One potential study might be to examine the utility of combining measures of asthma control along with another marker of inflammation and/or AHR to guide ICS therapy, and whether this might improve asthma control and future exacerbation rate better than either test alone. Even adding a more simple to measure biomarker such as blood eosinophils to routine review may prove useful. Another spin-off outcome from this could be compliance to ICS therapy, whereby keeping tighter control of both a biomarker and for example ACQ together might help patients understand their need for ongoing ICS therapy despite lack of symptoms.

The incidences of obesity and asthma are both increasing, necessitating a better understanding of how ICS effects differ between overweight and normal weight asthmatics. On the basis of this thesis' findings, several areas of future study are warranted. Firstly to examine whether it is indeed reduction in ICS delivery to the lung periphery through lung deposition imaging studies that is the main reason for reduced ICS efficacy in the obese asthma population. In addition, studies of small peripheral airway function in obese versus normal weight asthmatics would be useful both on static doses of ICS as well as the effects of incremental ICS in this regard. Leading on from this would be a study of the utility of specifically selecting extra-fine particle ICS in obese subjects on outcomes such as asthma control compared to standard size particle ICS i.e. whether potential better delivery of ICS leads to better outcomes in these patients.

One final area of potential future study relates to the use of beta-blockers in asthmatic subjects. This would not be for the treatment of asthma per se, but to examine how best to increase their use in patients who might need them, for example, when they might suffer comorbid ischaemic heart disease where the benefits of beta-blockade are dramatic. It is clear from this thesis that they are safe in carefully selected asthmatic patients controlled on ICS therapy. This would initially require a case-finding study for those patients with asthma who could/should be on beta-blockade for an alternative condition. Further study into how best to commence beta-blocker treatment would be needed on a large scale in order to challenge clinical equipoise, where beta-blockers are shunned in asthma for safety reasons that may no longer apply provided patients are controlled on ICS therapy.

To draw this discussion to a close, I have included a proposed algorithm using the findings from this thesis in order to potentially improve asthma control with regard to ICS dosing alone (Figure 30). On the basis of asthma control as measured by ACQ, where an individual's ICS treatment might not be altered on the basis of BTS/SIGN 2014 asthma guidelines[10], I have outlined serial measurements that could be employed in order of increasing complexity in order to attain total asthma control in a personalised manner. This algorithm would clearly need further study and validation, but this may be used as a starting point for that process through future research projects.

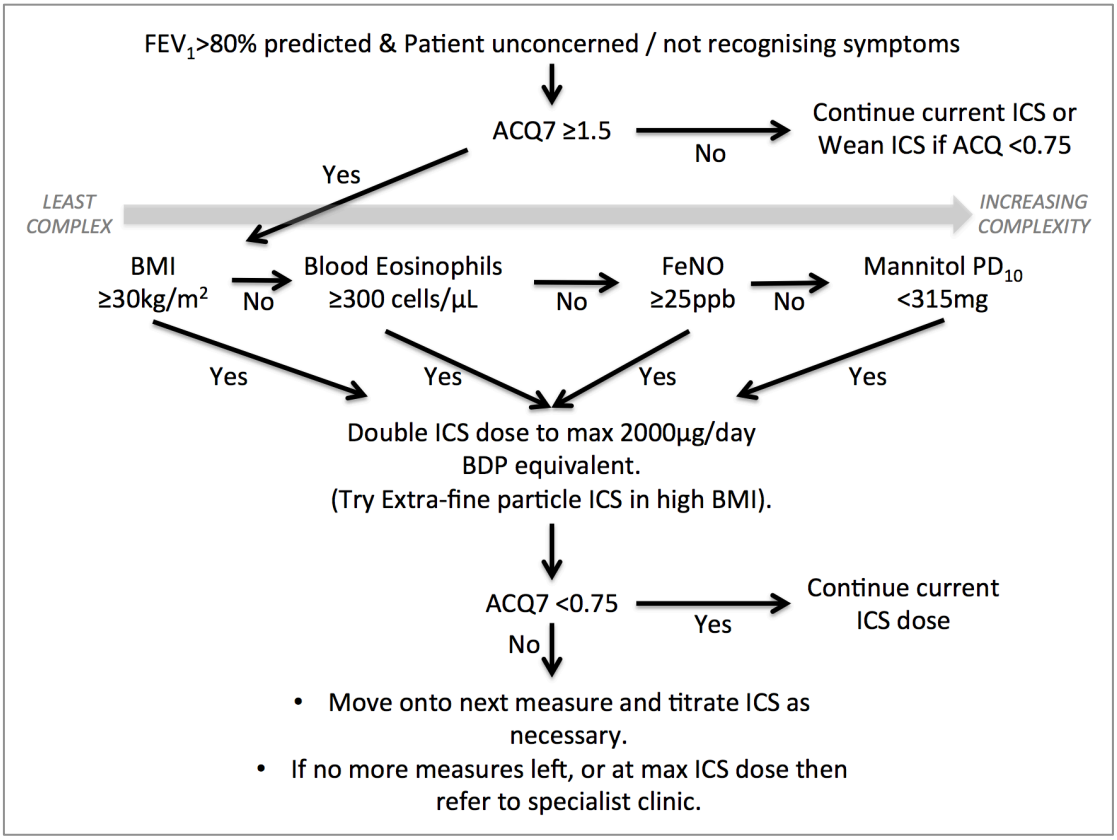

Figure 30. Proposed ICS dose titration algorithm.

This algorithm is purely theoretical and only for ICS dosing as per this thesis, where ICS dose may not be changed on the basis of BTS/SIGN 2014 asthma guidelines. It takes no account of other second line asthma therapies such as LABAs.

CONCLUSIONS

1. This thesis demonstrated a significant dose-response relationship to ICS using diurnal domiciliary FeNO measurement in asthmatics with elevated FeNO as a common inflammatory phenotype. These responses followed predictable exponential decay of FeNO that was significantly greater with higher ICS dosing. These findings translated into significant improvements in symptoms and asthma control in this selected phenotype despite the short study duration.

2. Overweight persistent asthmatics may have attenuated symptom and FeNO responses to stepwise ICS increments compared to normal weight asthmatic counterparts. There was no difference, however, between weight groups for either FEV_1 or methacholine responses at either dose. There was a trend to attenuated cortisol suppression in overweight subjects, suggesting the possibility of reduced bioavailability of ICS as the reason for reduced symptom and FeNO responses in this group.

3. Adding the beta-2 adrenoceptor inverse agonist propranolol to low dose ICS in persistent asthma provided no corticosteroid-sparing effect on histamine AHR and other inflammatory outcomes, compared to the significant improvements conferred by using a higher ICS dose. Salbutamol recovery post-histamine challenge was partially blunted and there was a small but significant fall in evening FEV_1 when adding propranolol to low dose ICS, but no deterioration in ACQ or AQLQ. One would not therefore advocate propranolol as a corticosteroid-sparing agent in persistent asthma. However, clinical equipoise might still be challenged as propranolol could perhaps be given at these doses

to asthmatic patients for its usual indications provided our strict safety criteria are adhered to in carefully selected patients controlled on ICS.

4. This thesis identified categories of mannitol AHR which are correlated to airway inflammation as measured by FeNO as well as to direct AHR using methacholine challenge in mild to moderate persistent asthmatics currently taking inhaled corticosteroids. This might provide a useful gauge of the degree of both airway inflammation and airway hyper-responsiveness in persistent asthmatics simultaneously. We also found that mannitol AHR is not associated with salivary ECP or airway calibre in these asthmatic subjects. These findings add weight to the argument that mannitol challenge is related to both local airway allergic inflammation and hyper-responsiveness in persistent asthma.

5. This thesis demonstrated further room for improvement in markers of inflammation and AHR, despite a plateau in the dose response to ICS for both asthma symptoms and pulmonary function, with both small and large particle ICS HFA-formulations and doses. This points to an unmet need of uncontrolled underlying inflammation in certain asthmatic patients, which may be a precursor to future loss of asthma control.

6. There were no significant differences in levels of sensitive markers of bone formation or resorption over 1 year, between groups using either low or high doses of ICS. Furthermore, there was no difference in approximated ratios of bone turnover with the higher ICS dose. These findings provide reassurance

that the higher ICS doses required to achieve control of asthmatic inflammation, over and above standard care, are safe with regard to the risk of steroid induced bone demineralisation.

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APPENDIX I – PEER REVIEWED OUTPUT

Peer Reviewed Publications

- The inverse agonist propranolol confers no corticosteroid sparing activity in mild to moderate persistent asthma. **Anderson WJ**, Short PM, Williamson PA, Manoharan A, Lipworth BJ. *Clin Sci (Lond)* 2014 Dec 1;127(11):635-43. doi: 10.1042/CS20140249.
- Influence of β2-adrenoceptor 16 genotype on propranolol-induced bronchoconstriction in patients with persistent asthma. **Anderson WJ**, Short PM, Manoharan A, Lipworth JL, Lipworth BJ. *Ann Allergy Asthma Immunol.* 2014 May;112(5):475-6. doi: 10.1016/j.anai.2014.02.016.
- The FeNOtype trial: Inhaled corticosteroid dose-response using domiciliary exhaled nitric oxide in persistent asthma. **Anderson WJ**, Short PM, Williamson PA, Lipworth BJ. *Chest.* 2012 Dec;142(6):1553-61. doi: 10.1378/chest.12-1310.
- Relationship of mannitol challenge to methacholine challenge and inflammatory markers in persistent asthmatics receiving inhaled corticosteroids. **Anderson WJ**, Lipworth BJ. *Lung*. 2012 Oct;190(5):513-21.
- Does body mass index influence responsiveness to inhaled corticosteroids in persistent asthma? **Anderson WJ**, Lipworth BJ. *Ann Allergy Asthma Immunol.* 2012 Apr;108(4):237-42. Epub 2012 Jan 21.
- Inhaled corticosteroid dose response in asthma: should we measure inflammation? **Anderson WJ,** Lipworth BJ. *Ann Allergy Asthma Immunol.* (Under rebutted peer review at time of final thesis submission, Oct 2016).

- Prospective follow-up of novel markers of bone turnover in persistent asthmatics exposed to low and high doses of inhaled ciclesonide over 12 months. **Anderson WJ**, McFarlane LC, Lipworth BJ. *J Clin Endocrinol Metab*. 2012;**97**:1929-36.

Oral Presentations

- The FeNOtype Trial: Effects of inhaled corticosteroids on asthmatic inflammation. **WJ Anderson**, PM Short, PA Williamson, BJ Lipworth. Scottish Thoracic Society Spring Meeting 2012 and British Thoracic Society Winter Meeting, London, 2012.
- Relationships of Mannitol Challenge to Methacholine Challenge and Inflammatory Markers in Persistent Asthmatics Receiving Inhaled Corticosteroids. **WJ Anderson**, BJ Lipworth. European Respiratory Society Annual Congress, Vienna, 2012.
- Does body mass index influence responsiveness to inhaled corticosteroids in persistent asthma? **W. Anderson**, B. Lipworth. Scottish Thoracic Society Winter Meeting 2011.

Poster Presentations

- The inverse agonist propranolol does not confer steroid-sparing activity in persistent asthma. **WJ Anderson,** PM Short, PA Williamson, A Manoharan, BJ Lipworth. European Respiratory Society Annual Congress, Munich, 2014.

- Effects of inhaled corticosteroids on asthmatic inflammation: The FeNOtype Trial. **WJ Anderson**, PM Short, PA Williamson, BJ Lipworth. European Respiratory Society Annual Congress, Vienna, 2012.
- Prospective follow-up of novel bone turnover markers in asthmatics exposed to low or high doses of inhaled ciclesonide over one year. **WJ Anderson,** LC McFarlane, BJ Lipworth. European Respiratory Society Annual Congress, Vienna, 2012.

APPENDIX II – ASTHMA QUESTIONNAIRES

Asthma Control Questionnaire

Please answer questions 1 – 6

Circle the number of the response that best describes how you have been during the past week.

1. On average, during the past week, how often were you woken by your asthma during the night?	0 Never 1 Hardly ever 2 A few times 3 Several times 4 Many times 5 A great many times 6 Unable to sleep because of asthma
2. On average, during the past week, how bad were your asthma symptoms when you woke up in the morning?	0 No symptoms 1 Very mild symptoms 2 Mild symptoms 3 Moderate symptoms 4 Quite severe symptoms 5 Severe symptoms 6 Very severe symptoms
3. In general, during the past week, how limited were you in your activities because of your asthma?	0 Not limited at all 1 Very slightly limited 2 Slightly limited 3 Moderately limited 4 Very limited 5 Extremely limited 6 Totally limited
4. In general, during the past week, how much shortness of breath did you experience because of your asthma?	0 None 1 A very little 2 A little 3 A moderate amount 4 Quite a lot 5 A great deal 6 A very great deal

5.	In general, during the past week, how much time did you wheeze?	0 Never 1 Hardly any of the time 2 A little of the time 3 A moderate amount of the time 4 A lot of the time 5 Most of the time 6 All the time
6.	On average, during the past week, how many puffs/inhalations of short-acting bronchodilator (e.g. Ventolin/Bricanyl) have you used each day? (If you are not sure how to answer this question, please ask for help)	0 None 1 1-2 puffs/inhalations most days 2 3-4 puffs/inhalations most days 3 5-8 puffs/inhalations most days 4 9-12 puffs/inhalations most days 5 13-16 puffs/inhalations most days 6 More than 16 puffs/inhalations most days

To be completed by a member of the clinic staff

7.	FEV_1 pre-bronchodilator:…… FEV_1 predicted:…….. FEV_1 %predicted:…….. (Record actual values on the dotted lines and score the FEV1 %predicted in the next column)	0 >95% predicted 1 95-90% 2 89-80% 3 79-70% 4 69-60% 5 59-50% 6 <50% predicted

Mini Asthma Quality of Life Questionnaire

Please complete all questions by circling the number that best describes how you have been during the last 2 weeks as a result of your asthma.

IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:

		All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time
1	Feel short of breath as a result of your asthma?	1	2	3	4	5	6	7
2	Feel bothered by or have to avoid dust in the environment?	1	2	3	4	5	6	7
3	Feel frustrated as a result of your asthma?	1	2	3	4	5	6	7
4	Feel bothered by coughing?	1	2	3	4	5	6	7
5	Feel afraid of not having your asthma medication available?	1	2	3	4	5	6	7
6	Experience a feeling of chest tightness or chest heaviness?	1	2	3	4	5	6	7
7	Feel bothered by or have to avoid cigarette smoke in the environment?	1	2	3	4	5	6	7
8	Have difficulty getting a good night's sleep as a result of your asthma?	1	2	3	4	5	6	7
9	Feel concerned about having asthma?	1	2	3	4	5	6	7
10	Experience a wheeze in your chest?	1	2	3	4	5	6	7

IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:

		All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time
11	Feel bothered by or have to avoid going outside because of weather or air pollution?	1	2	3	4	5	6	7

HOW LIMITED HAVE YOU BEEN DURING THE LAST 2 WEEKS DOING THESE ACTIVITIES AS A RESULT OF YOUR ASTHMA?

		Totally limited	Extremely limited	Very limited	Moderate limitation	Some limitation	A little limitation	Not at all limited
12	Strenuous activities (such as hurrying, exercising, running up stairs, sports)	1	2	3	4	5	6	7
13	Moderate activities (such as walking, housework, gardening, shopping, climbing stairs)	1	2	3	4	5	6	7
14	Social activities (such as talking, playing with pets / children, visiting friends / relatives)	1	2	3	4	5	6	7
15	Work-related activities* (tasks you have to do at work)	1	2	3	4	5	6	7

*If you are not employed or self-employed, these should be tasks you have to do most days.

Mini-AQLQ Domain Code: Symptoms (1, 4, 6, 8, 10); Activity limitations (12, 13, 14, 15); Emotional function (3, 5, 9); Environmental stimuli (2, 7, 11).

Asthma Control Test

Take this test if you are 12 years or older. Share the score with your healthcare provider.

Step 1: Write the number of each answer in the score box provided.
Step 2: Add up each score box for the total.
Step 3: Take the completed test to your healthcare provider to talk about your score.

If your score is 19 or less, your asthma symptoms may not be as well controlled as they could be. No matter what the score, bring this test to your healthcare provider to talk about the results.

						SCORE
1.	In the past 4 weeks, how much of the time did your asthma keep you from getting as much done at work, school or at home?					
	All of the time [1]	Most of the time [2]	Some of the time [3]	A little of the time [4]	None of the time [5]	……
2.	During the past 4 weeks, how often have you had shortness of breath?					
	More than once a day [1]	Once a day [2]	3 to 6 times a week [3]	Once or twice a week [4]	Not at all [5]	……
3.	During the past 4 weeks, how often did your asthma symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) wake you up at night or earlier than usual in the morning?					
	4 or more nights a week [1]	2 to 3 nights a week [2]	Once a week [3]	Once or twice [4]	Not at all [5]	……
4.	During the past 4 weeks, how often have you used your rescue inhaler or nebulizer medication (such as salbutamol)?					
	3 or more times per day [1]	1 or 2 times per day [2]	2 to 3 times per week [3]	Once a week or less [4]	Not at all [5]	……
5.	How would you rate your asthma control during the past 4 weeks?					
	Not controlled at all [1]	Poorly controlled [2]	Somewhat controlled [3]	Well controlled [4]	Completely controlled [5]	……
					TOTAL:	……